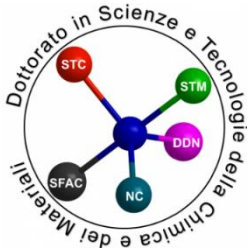

Università degli Studi di Genova

Doctoral School in Sciences and Technologies of Chemistry and Materials

Ph.D. in Chemical Sciences and Technologies

XXXII CYCLE

Development of novel bio-based building blocks and their application in organic synthesis and materials science

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Advisor: **Prof. Renata Riva**

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Abstract

In the progression of modern society, the inevitable exhaustion of fossil resources becomes an increasingly concerning matter. These resources are not only the basis of the energy production of the world but also the precursor for many important platform chemicals. Therefore, development of green and renewable alternatives to the chemicals used in the current industry has become necessary. Within this context, the aim of the present thesis was the development of new efficient methodologies for obtaining high added-value products starting from biomass, a renewable source. The bio-based building blocks obtained were subsequently employed for the synthesis of polyfunctionalized chemicals and of polymers.

The first part of the thesis focuses on the utilization of a bio-based *meso* diol for the synthesis of sugar derived structures, through the coupling of biocatalysis and multicomponent reaction (MCRs). These two different approaches are able to satisfy the requirements of an ideal approach, regarding selectivity (stereocontrol), efficiency and sustainability and their union with renewable feedstocks in a single integrated general strategy represents a powerful tool to achieve a sustainable synthesis. In particular, the work focused on the synthetic elaboration of the chiral molecules obtained through enzymatic desymmetrization to give enantiomerically pure building blocks to be used in diastereoselective Passerini reactions. In order to establish the synthetic usefulness of this new method, we demonstrated its application to the diversity-oriented synthesis of chiral, bio-based, oxygen heterocycles and to the target-oriented synthesis of Bengamides, a wide family of natural products of marine origin.

The second part of the thesis is dedicated to the synthesis of a lactam- based monomer derived from bio-based starting materials and its subsequent polymerization. The project involved the synthesis and polymerization of 3-methylene-2-piperidone (3M2Pip) monomers, targeting the synthesis of well-defined hydroxyl end functional P(3M2Pip), i.e. HO-P(3M2Pip). Once obtained end-functionalized 3M2Pip-based polymers we will use them as a macro-initiator for the synthesis of degradable polyester second blocks, applicable in drug delivery polymer materials.

Table of contents

List of Tables

List of Figures

List of Schemes

List of Publications

G. Vitali Forconesi, L. Banfi, A. Basso, C. Lambruschini, L. Moni, and R. Riva, "Synthesis of polyoxygenated heterocycles by diastereoselective functionalizations of a bio-based chiral aldehyde exploiting the Passerini reaction", *Molecules*, **2019**, submitted

G. Vitali Forconesi, L. Moni, R. Riva et al. "Chemoenzymatic Total Synthesis of 4-epi-Bengamide E: a fruitful example of combination of renewable sources, Multicomponent Reaction, and Biocatalysis" manuscript under preparation.

List of Abbreviations

Å	Ångström
ABCN	1,1′-Azobis(cyclohexanecarbonitrile)
Ac	Acetyl
AcOH	Acetic acid
AcOEt	Ethyl acetate
ACVA	4,4′-Azobis(4-cyanovaleric acid)
AIBN	Sodium bis(2-methoxyethoxy)aluminium hydride
AMANO PS	Lipase from *Pseudomonas cepacia*
BAIB	(Diacetoxyiodo)benzene
Bn	Benzyl
Boc	*tert*-butyloxycarbonyl
*n*BuLi	*normal*- butyl lithium
*t*Bu	*tert*-butyl
Bz	Benzoyl
CAL- B	Lipase from *Candida Antarctica*
CAN	Cerium (IV) ammonium nitrate
CTA	Chain transfer agent
DCC	*N*,*N*‘-dicyclohexylcarbodiimide
DCM	Dichloromethane
DCU	*N*,*N*‘-dicyclohexylurea
DDI	Distilled deionized (water)
DIBAL-H	Diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMP	*2,2*-dimethoxypropane
DMSO	Dimethyl sulfoxide
DMF	*N*,*N*-dimethylformamide
DOS	Diversity-oriented synthesis
d.r.	Diastereomeric ratio
e.e.	Enantiomeric excess
eq.	Equivalent
et al.	*Et alii* (and others)
Et	Ethyl
ETP	Petroleum ether
FRP	Free Radical polymerization
GC	Gas chromatography
HOMO	Highest occupied molecular orbital
HMF	Hydroxymethyl furfural
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectrometry
Hz	Hertz
IMCR	Isocyanide based multicomponent reaction
IR	Infrared spectroscopy
LiHMDS	Lithium hexamethyldisilazide
LRP	Living radical polymerization
LUMO	Lowest unoccupied molecular orbital
3M2P	3-methylene-2-pyrrolidone
3M2Pip	3-methylene-2-piperidone

Me	Methyl
MetAP	Methionine aminopeptidase
m.p.	Melting point
MCR	Multicomponent reaction
NaHMDS	Sodium hexamethyldisilazide
NMR	Nuclear magnetic resonance
Nu	Nucleophile
NVP	N-vinyl pyrrolidone
P-3CR	Passerini three component reaction
P(3M2P)	Poly(3-methylene-2-pyrrolidone)
P(3M2Pip)	Poly(3-methylene-2-piperidone)
PMP	*para*-methoxyphenyl
PPL	Lipase from *Porcine Pancreas*
*i*Pr	*iso*-propyl
*n*Pr	*normal*-propyl
PVP	Poly(vinyl pyrrolidone)
RAFT	Reversible Addition Fragmentation chain Transfer
Red-Al	Sodium bis(2-methoxyethoxy)aluminium hydride
R*f*	Retention factor
TOS	Target- oriented synthesis
p-TsOH	*para*-toluenesulfonic acid
Pyr	Pyridine
RCM	Ring closing metathesis
r.t.	Room temperature
S-PPL	Lipase from *Porcine Pancreas* immobilized on celite
TBAD	*tert*-butyl azodicarboxylate
TBAF	Tetra-*n*-butylammonium fluoride
TBDMS/ TBS	*tert*-butyldimethylsilyl
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy radical
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
TOS	Target- oriented synthesis
UV-VIS	Ultraviolet-visible

Chapter 1

General Introduction

In this chapter, a general overview of the concepts of Bio-economy and Green Chemistry will be provided. These two philosophies afford a general guideline for the use of renewable sources, such as biomass, for chemicals production.

CHAPTER 1.
GENERAL INTRODUCTION

1.1. Overview

Nowadays fine chemicals industries are still mostly based on fossil fuels reservoir. In the progression of modern society, the inevitable exhaustion of fossil resources becomes an increasingly concerning matter. These resources are not only the basis of the energy production of the world but also the precursor for many important platform chemicals. With the consumption of fossil resources on the horizon, alternatives have to be developed.

In this context, the development of a Green Chemistry plays an essential role. Based on the Twelve Principles, proposed by Anastas and Warner[1], Green Chemistry has been defined as "*efficient use of (preferably renewable) raw materials, which eliminates waste, avoids the use of toxic and/or hazardous reagents and eliminates solvents in the manufacture and application of chemical products"*.

1. Prevent waste	7. Use of renewable feedstocks
2. Atom economy	8. Reduce derivatives
3. Less hazardous synthesis	9. Catalysis (vs. stoichiometric)
4. Design benign chemicals	10. Design for degradation
5. Benign solvents and auxiliaries	11. Real-time analysis for pollution prevention
6. Design for energy efficiency	12. Inherently benign chemistry for accident prevention

Table 1.1 Twelve Principles of Green Chemistry

More recently, a mnemonic, PRODUCTIVELY, has been proposed by Poliakoff et al.[2] which captures the spirit of the twelve principles of Green Chemistry.

Although attention continues to be focused on waste minimisation and avoiding the use of toxic and/or hazardous reagents and solvents, the last decade has witnessed a growing emphasis on using starting materials derived from renewable sources instead of non-renewable fossil feedstock – crude oil, coal and natural gas – for the production of fuels, bioplastics and chemicals. These materials wholly or partly derived from renewable, biological sources, may be termed as *bio-based products*[3].

Bio-based products are included in concepts of *bio-based economy* and circular economy, which have received support from policymakers and advisors at national and supranational levels. Bio-economy is a new concept that officially came into light for discussion at the beginning of the 21st century. However, it was not until the second decade of the 21st century that it has attracted a great interest of scientists shaping development strategies. According to European Commission[4], "*The bio-economy comprises those parts of the economy that use renewable biological resources from land and sea such as crops, forests, fish, animals and micro-organisms to produce food, materials, and energy*".

Utilization of bio-based building blocks derived from renewable resources and of green processes is the principle which bio-economy focuses on to reach the goal of sustainable global development.

1.2. Biomass for the production of fine chemicals and biopolymers

From a chemical point of view the most important renewable resource is biomass. The term biomass is defined as any organic matter that is available on a renewable basis, including dedicated energy crops and trees, agricultural food and feed crop residues, aquatic plants, wood and wood residues, animal wastes and other waste materials. Biomass represents an abundant, widely available and sustainable carbon renewable resource and the perfect equivalent to petroleum for the production of fuels and fine chemicals with net-zero carbon emission. This material is generated from available atmospheric CO_2, water and sunlight through biological photosynthesis. Through bio-refinery, each of the different components of given natural resource is isolated by chemical and biochemical means with the aim of turning them into useful products. Thus, interesting chemicals, monomers and polymers could replace progressively petrol-based counterparts. These new bio-based products are not necessarily identical to those produced starting from oil; they could be in principle even superior, for example for biodegradability.

Broadly, biomass can be categorized as first, second, third and fourth generation. The biomass-based chemicals and fuels generally are produced by sugar and starch-containing crops such as beet, corn and grain, the so-called first-generation biomass. These crops are also used for food and animal feed, resulting in an undesirable competition between food and chemicals. It is therefore important the search for alternative non-edible feedstocks. Second generation biomass consists of non-food parts of crops and other forms of lignocellulosic biomass such as wood, grasses and municipal solid wastes. Finally, third and fourth generation can be used as they include micro-algae and microorganism derived feedstocks.

Lignocellulosic biomass materials are receiving increased attention as a renewable, economical, and abundant alternative to fossil resources for the production of bio-based chemicals and polymers[5]. It represents the most abundant and renewable biomass on earth, it is significantly cheaper than crude oil and it can be produced quickly and at a lower cost than other agriculturally important feedstocks. A distinct advantage of this biomass is that its utilization for chemical, material or biofuel production does not directly interfere with food supplies, as lignocellulosic material is non-edible by humans.

The three main components of lignocellulose are cellulose (40–50%), hemicellulose (25–35%) and lignin (15–20%). The composition of the three main fractions may vary according to plant type, varieties, part and maturity. Other components are fatty acids, lipids, proteins.

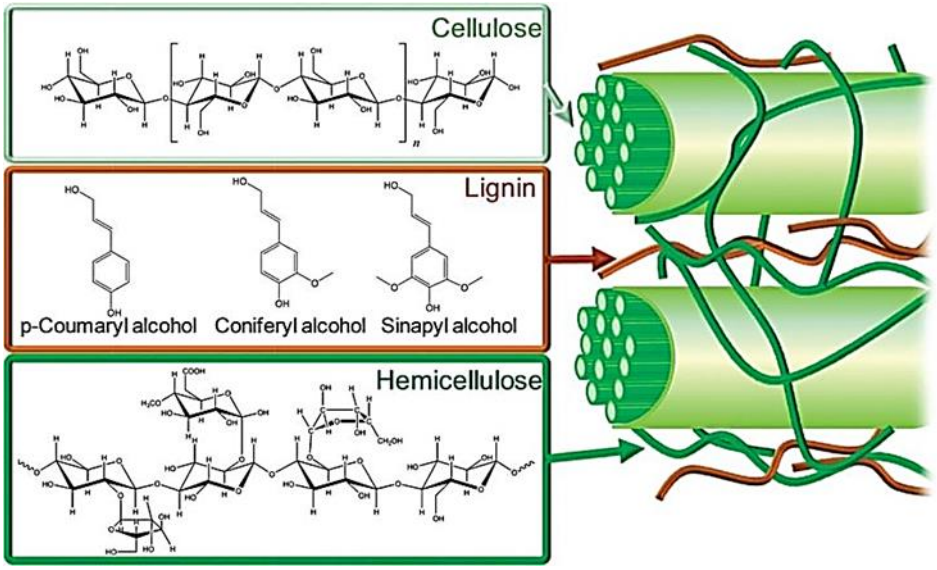
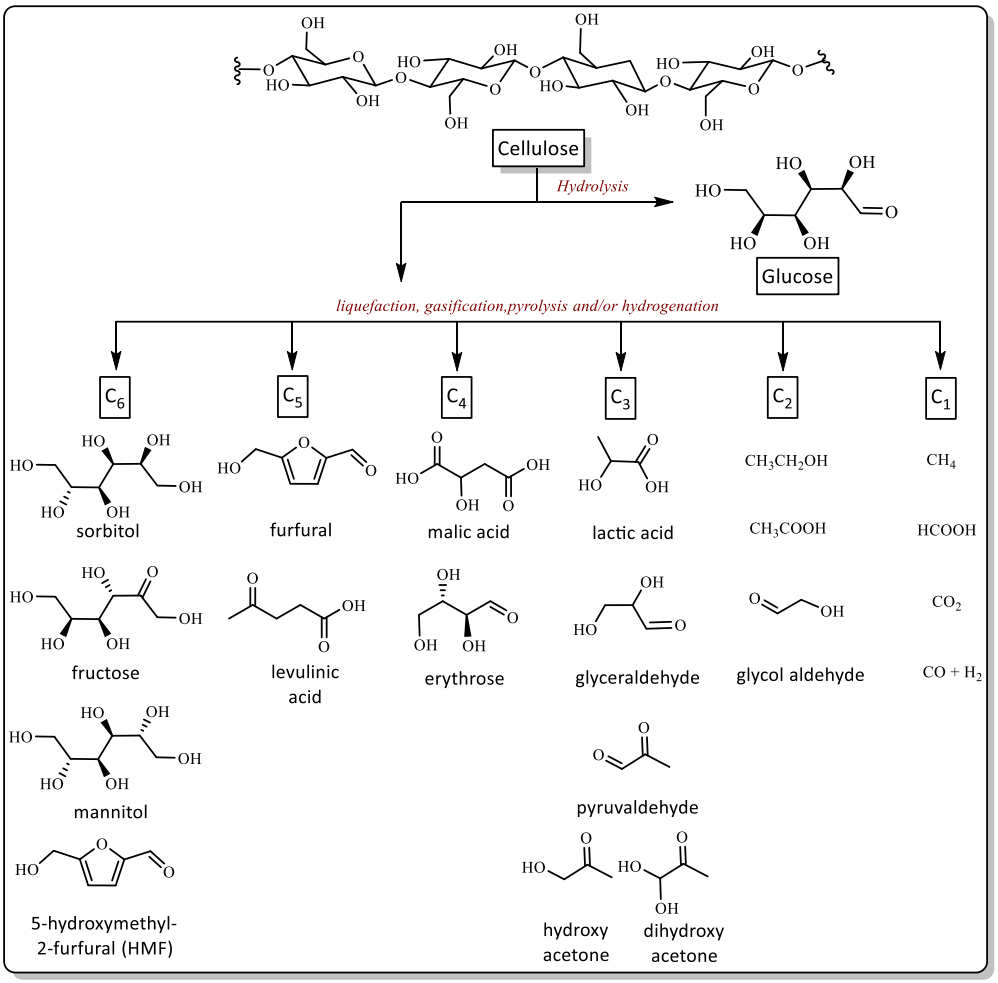


Figure 1.1 Schematic representation of woody biomass composition
Taken from *Alonso et al.* [6]

Cellulose is a non-branched water-insoluble polysaccharide consisting of D-glucose subunits linked by β-(1→4) glycosidic bond. Hemicellulose is a polymeric material, although lower in molecular weight than cellulose, consisting of C6-sugars (glucose, mannose and galactose) and C5-sugars (mainly arabinose and xylose). Lignin is an amorphous heteropolymer constructed of phenolic monomer units linked in a three-dimensional structure.

Among the different components of lignocellulosic biomass, carbohydrates, in particular, are promising because they are inexpensive, readily available, provide great stereochemical diversity and form by far the largest natural source of carbon. Many types of bio-based building blocks can be produced or extracted from carbohydrates through biological, chemical, thermal, or mechanical treatments. These building

blocks can subsequently be converted into a wide spectrum of valuable chemicals and materials.



Scheme 1.1 Platform chemicals from cellulose
Taken from *Donate* [7]

Currently, the industrial production of bio-based compounds mainly involves polymers. Synthetic polymers, or plastics, are essential materials to modern society. Industrial polymerization technologies make it possible to produce versatile polymers with highly tunable properties and a broad range of applications. No other class of materials can have such diverse properties and versatile applicability. The polymers that currently dominate the market (polyolefins such as polyethylene and polypropylene, or polyesters such as polyethylene terephthalate) derived from non-renewable oil and gas resources, are environmentally persistent, costly to degrade, which make them unsustainable. Thus, the development of high-performance polymers and novel monomers from renewable feedstocks is a crucial step toward a global economy that is less dependent on fossil resources and has been a field of increasing study.

In the fine chemicals area, there is also increasing interest in Green chemistry. Up to now, research has been focused mainly on the use of biomass for the production of large quantities of low-cost commodities, such as bioplastics, whereas the preparation of high added-value compounds, such as Active Pharmaceutical Ingredients, potential new drugs, nutraceutical additives, cosmetics ingredients and so on has been much less investigated so far. Thanks to the high value of these bio-based products, also natural starting material obtained in relatively small quantities may be exploited. Within this context, a whole new chemistry has to be developed, alternative to the classic organic synthesis, which starts mainly from mineral oil.

1.3. Aim and outline of this thesis

Owing to the importance of biomass, the aim of the present thesis was the development of new efficient methodologies for obtaining high added-value products starting from renewable sources. The bio-based building blocks obtained were subsequently employed for the synthesis of polyfunctionalized chemicals and polymers.

In *Chapter 1*, a general overview of the concept of Green Chemistry and bio-economy is provided. Moreover, the importance of using biomass as a source of bio-based starting materials is illustrated.

The results obtained are then organized in two main sections.

The first section (**Part A**), which includes *Chapters 2*, *3* and *4*, will deal with two different projects developed within the BioOrganic Chemistry Group[8] at the Department of Chemistry and Industrial Chemistry of the University of Genova, under the supervision of Prof. Renata Riva. The group is deeply involved in Diversity Oriented Synthesis of libraries of drug-like molecules, especially using multicomponent reactions (MCRs). Recently, the group is becoming active in new fields, such as flow chemistry and photoactivated reactions and in the chemical contribution to bio-economy as well.

These Chapters will discuss the application of bio-based building blocks to the organic synthesis of sugar derived structures. Particularly, in *Chapter 2*, the advantages of coupling biocatalysis, multicomponent reactions and the use of bio-based building blocks will be described. In *Chapter 3* and *4* the diversity-oriented synthesis of chiral, bio-based, oxygen heterocycles and the target-oriented synthesis of Bengamides, a new class of antitumor natural products of marine origin, will be explored.

The second section (**Part B**), which includes *Chapter 5*, will be focused on the project developed in the Free Radical Group[9] at Stellenbosch University (South Africa) under the supervision of Prof. Bert Klumperman and Dr. Rueben Pfukwa,

during the period (6 months) I spent as visiting Ph.D. student. The research of this group focusses on the use of reversible deactivation radical polymerization (RDRP) as a versatile tool to create carefully designed polymers with complex architectures that can be employed in the biomedical field such as hydrogels or drug delivery systems.

This Chapter will describe a further application of bio-based building blocks in the field of materials science. Particularly, after a brief introduction on the BIODEST Project, the synthesis of a novel lactam-based monomer and its use for preliminary polymerization reactions will be presented.

1.4. Bibliography

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DCCI

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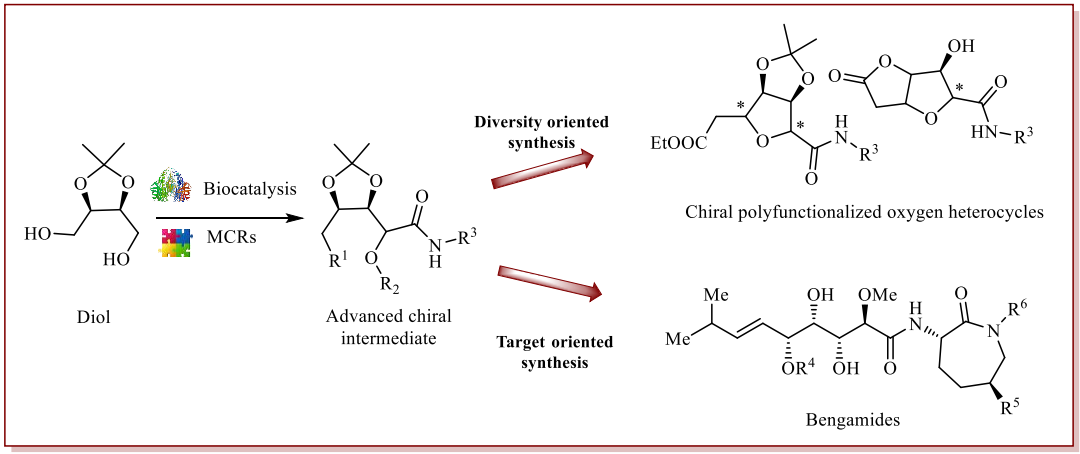
Part A

Bio-based building blocks in organic synthesis

In this section, the first part of my doctoral research work, carried out in the BioOrganic Chemistry Group at the University of Genova, will be discussed.

I worked on two different projects united by the possibility of using a bio-based building block, diol, derived from biomass, to prepare high added-value compounds, through the coupling of biocatalysis and multicomponent reactions (MCRs). The validity of our synthetic strategy was demonstrated by the application to the diversity-oriented synthesis of polyfunctionalized oxygen heterocycles and to the target-oriented synthesis of Bengamides.



CHAPTER 2.
BIOCATALYSIS - MCRs COUPLING

2.1 Introduction

In the past century, the main purpose of organic chemists was to find pathways for the synthesis of many complex molecules. Since then, many novel methodologies have been discovered, making concrete the possibility to obtain almost every needed molecule. However, over the past few decades, the purpose has been shifted to how these complex molecules can be prepared. Chemists have to be progressively more devoted to the achievement of an ideal and "greener" synthesis[1]. According to P. A. Wender and R. J. Ternansky[2] "*an ideal synthesis is generally regarded as one in which the target molecule (natural or designed) is prepared from readily available inexpensive starting materials in one simple, safe environmentally acceptable, and resource-effective operation that proceeds quickly and in quantitative yield*".

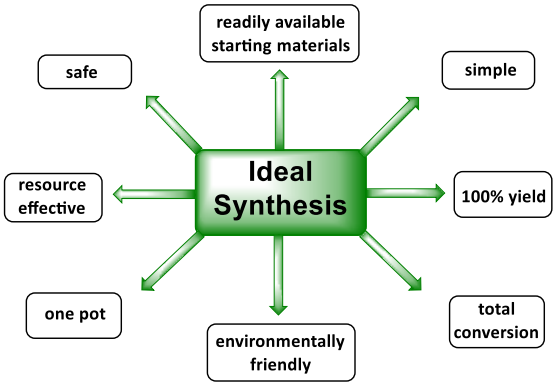


Figure 2.1 Features of an ideal synthesis
Taken from *Dõmling and Ugi*[3]

Within this context, coupling biocatalysis with multicomponent reactions is of particular interest. These two different approaches are able to satisfy the requirements of an ideal approach, regarding selectivity (stereocontrol), efficiency and sustainability[4].

In multicomponent reactions (MCRs) often multiple covalent bonds and several stereocentres are formed in a single reaction step with a high tolerance of different functional groups. Consequently, the use of protection groups can be avoided, which reduces the number of reaction steps significantly. Moreover, since multicomponent reactions are based on the condensation of three or more substrates in a single product, a relevant chemical complexity and diversity level is inserted in the final products, as the structure of the reacting substrates can be varied at will. This synthetic methodology is highly convergent and efficient and responds to the criteria

of atom[5] and step economy. In addition, MCRs are usually experimentally simple to perform and need mild conditions. Considering all these features, it is not surprising that MCRs are widely recognized to be more sustainable synthetic methodologies compared to classical multistep synthesis (*Figure 2.2*).

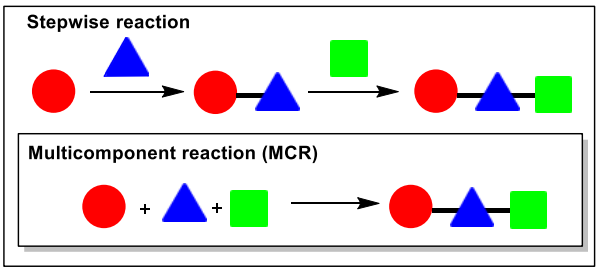


Figure 2.2 Comparison between a stepwise and a multicomponent approach.

Recently, Orru [6] and co-workers compared the general features of MCRs with Green Chemistry principles, demonstrating their close correlation, as summarized in *Table 2.1*.

Table 2.1 Correlation between Green Chemistry principles and MCRs features

Green Chemistry Principles	**MCRs features with related advantages and drawbacks**
Waste Production	✓ Convergence and step economy allow for solvent reduction ✓ Low MW and innocuous by-products
Atom Economy	✓ At least 80% of atom economy × Synthesis of isocyanide is not green (IMCRs)
Less Hazardous Synthesis	✓ Reagents and by-products are in most cases not hazardous
Design for Safer Products	✓ Not relevant (product not process)
Benign Solvents and Auxiliaries	✓ Simplified product isolation and purification (crystallization instead of chromatography) ✓ Step economy allows solvent reduction
Energy Efficiency	✓ Usually mild conditions ✓ Compatibility with microwave heating and flow technologies
Renewable Feedstocks	✓ Valuable use of renewable starting materials towards molecular complexity
Reduce Use of Derivatives	✓ Chemoselectivity and tolerability toward different functional groups; in most cases protecting groups are not required
Catalysis	✓ Some examples of organocatalysts and enzymes used × Stereocontrol through asymmetric catalysis is still an open issue
Design for Degradation	✓ Not relevant (product not process)

Real-Time Analysis for Pollution Control	✓ Possibility of coupling MCRs with flow conditions
Inherently Safer Processes	✓ Mild and safe conditions

Although the increasing number of applications of MCRs in target-oriented[7] and diversity-oriented synthesis[8], they suffer from a main limitation, which dramatically restricts their purpose: the overall poor stereocontrol in most of these reactions. This is a huge limitation in particular for the synthesis of Active Pharmaceutical Ingredients, in which the obtainment of a single enantiopure stereoisomer is of vital importance. To overcome this weakness there are two different possibilities[9]:

1) Perform asymmetric catalytic MCRs starting from achiral substrates;
2) Perform the reaction on chiral enantiomerically pure substrates taking advantage of the substrate controlled diastereoselection achieved during the MCR step.

The first approach benefits from the use of only small amounts of chiral catalysts. However, catalytic asymmetric methods to control the stereochemical outcome of MCRs are scarce. This is probably due to the fact that it is a very challenging result to obtain, in particular in isocyanide-based multicomponent reactions (IMCRs), in which one of the substrates is a molecule carrying isocyanide functional group. In the second approach, the chiral compound could be used both as a chiral auxiliary or as an effective substrate of the reaction. The chiral auxiliary approach results to be disadvantageous because it doesn't follow the atom economy principle, it introduces more manipulation in the procedure and wastes a possibility to introduce an additional diversity input. For this reason, the use of a chiral substrate is considered preferable for both atom and step economy together with the introduction of an additional diversity input. These can be derived easily and economically from the chiral pool (i.e., amino acids or sugars) but the available structures are quite limited. Consequently, methods for the generation of optically pure compounds to employ as MCRs inputs are required.

Within this context, biocatalysis is a promising method. Biocatalysts are among the most powerful tools in organic synthesis because of their great advantages[10]. Enzymes are characterized by impressively high efficiency and selectivity and they can catalyse a broad spectrum of reactions, also some impossible to emulate in classical organic chemistry. Biocatalysis has many attractive features in the context of Green Chemistry: mild reaction conditions (pH, temperature and solvent), environmental compatibility, biodegradability of enzymes. Moreover, the use of enzymes generally avoids the protection and deprotection steps required in traditional organic syntheses. This affords shorter processes, generate less waste and is environmentally more attractive than conventional routes[11]. Biocatalytic processes can be performed with isolated enzymes or as whole-cell biotransformations. Isolated

enzymes have the advantage of not being contaminated with other enzymes present in the cell, while the use of whole cells is less expensive since it avoids separation and purification of the enzyme.

Two main combinations of biocatalysis with MCRs are possible[12]:

(A) Biocatalysis can provide the enantiopure chiral inputs to use in MCRs.
(B) MCR was performed, followed by a kinetic resolution of the racemic products or a desymmetrization of *meso*/*prochiral* MCR adducts.

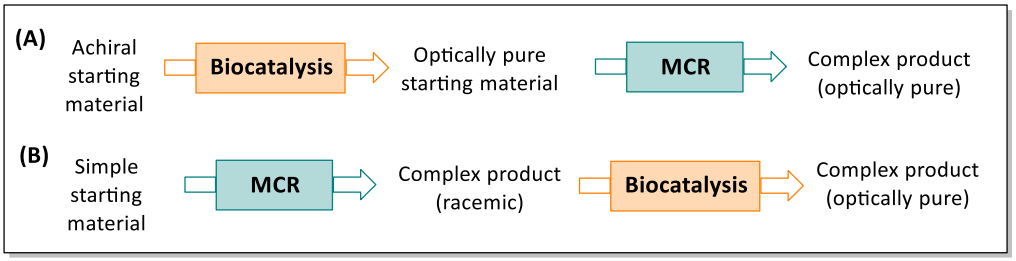


Figure 2.3 Combination of biocatalysis and MCRs

The main scope of this Part A was, therefore, to further investigate this field. In particular, my work is focused on the possibility of using biocatalysis for the synthesis of enantiopure chiral substrates that can be used to direct the stereochemical outcome of Passerini multicomponent reaction starting from bio-based compounds.

In the following paragraphs, the enzymatic methodology and multicomponent reaction exploited in this work will be described in detail. Finally, the synthetic plan elaborated during this thesis will be mentioned.

2.2 IMCRs: Passerini reaction

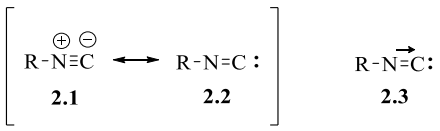
A large and important class of MCRs are the Isocyanide - based Multi-Component Reactions (IMCRs), which are widely used nowadays to efficiently prepare complex molecules, both in target-oriented synthetic projects and in combinatorial and diversity-oriented applications. The importance of IMCRs deals with the great potential of isocyanide molecule[13].

2.2.1 Structure and reactivity of isocyanide

Isocyanides are one of the most intriguing functional groups in organic chemistry. They are the only class of stable organic molecules with a formally divalent carbon atom, which can react with electrophiles and nucleophiles, participates in radical reactions[14] or act as a ligand to metal centres[15]. The attractions of these functional groups are offset by several disadvantages. These compounds are generally

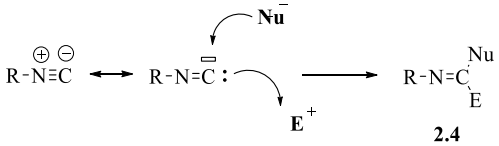
well known in the synthetic organic community but mostly because of their strong repulsive odour, described from Gautier[16] as "reminiscent of artichokes and phosphorus at the same time", rather than their unique reactivity. This class of compounds showed, however, to have only slight toxicity, apart from few exceptions. Moreover, few isocyanides are available from major commercial suppliers and they are typically not simple to prepare.

The reactivity of isocyanides can be explained with the representation of the electronic structure. Isocyanides are considered as resonance forms between divalent carbon form and zwitterion (*Scheme 2.1*). The carbene-like reactivity is reflected in the resonance structure **2.2**, while the linearity of the bond is represented by the resonance structure **2.1**. However, structure **2.3** with a donor-acceptor double bond and an electron lone pair at the carbon atom, may be more appropriate to describe the isocyanide chemistry.



Scheme 2.1 Resonance structures of isocyanide

Synthetically, the most important property of isocyanides is their unique ability to react simultaneously with nucleophiles and electrophiles at the isocyanide terminal carbon atom (α-addition) in a single reaction[3], leading to adducts such as **2.4** (*Scheme 2.2*). Most other functional groups react with nucleophiles and electrophiles at different centres. Only carbenes and carbon monoxide share this property with isocyanides.



Scheme 2.2 Reaction of isocyanide with nucleophile and electrophile

The difference between nitriles and isocyanides functions can be easily revealed by the comparison of the frontier orbitals (*Figure 2.4*). On isocyanide, a nucleophile attacks the carbon atom as it has the largest coefficient in the LUMO (π*) orbital. Moreover, an electrophile interacts with the HOMO (σ) orbital, which is only developed on the same terminal atom. So, both nucleophile and electrophile attacks will occur on the terminal carbon. Nitriles, in contrast, are attacked by nucleophiles at the carbon atom (higher π* orbital coefficient) and by electrophiles at the nitrogen atom (higher π orbital coefficient), displaying thus a "classical" reactivity at two centres.

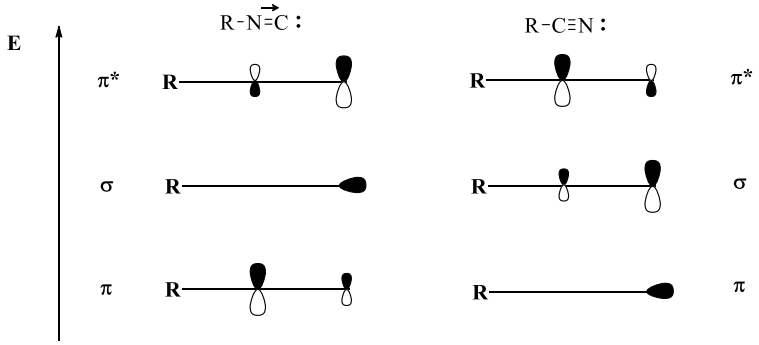
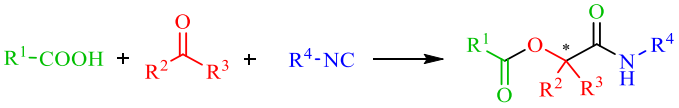


Figure 2.4 Frontier orbitals of isocyanide and cyanides.
Taken from *Dömling, and Ugi*[3]

Due to their ability to engage in multiple-bond forming reactions, isocyanides have found wide applications in multicomponent chemistry.

2.2.2 Passerini reaction (P-3CR)

Passerini reaction, discovered by Mario Passerini in 1921 in Italy[17], is the first example of a multicomponent reaction with an isocyanide as basic moiety. It involves the reaction of a carbonyl compound (a ketone or aldehyde), an isocyanide, and a carboxylic acid to afford α-acyloxycarboxamide scaffolds, under mild conditions and with high level of atom economy.

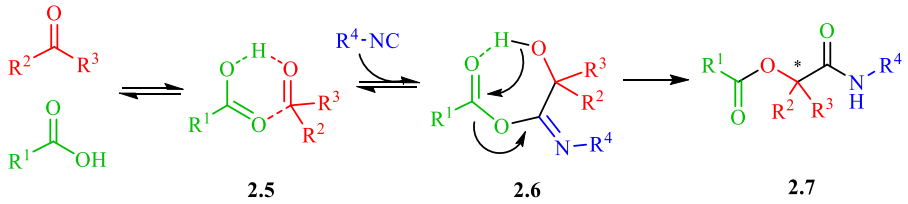


Scheme 2.3 Passerini reaction

In the last decades, in order to expand the scope of this procedure, different approaches such as intramolecular modification or post-condensation transformations[18], have been explored.

2.2.2.1 Mechanism

The mechanism of Passerini reaction has been widely discussed[19]. The main problem is that intermediates from a MCR are typically not isolated, thus causing difficulties to obtain a more precise mechanism proposal for the transformation. The classical accepted mechanism is depicted in *Scheme 2.4* [20].

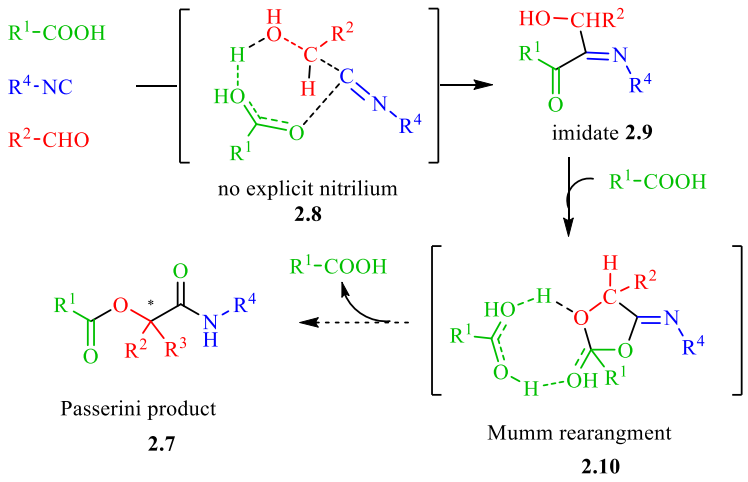


Scheme 2.4 Classical mechanism

At first, the carboxylic acid and the carbonyl compound form a six-membered adduct (**2.5**) stabilized via hydrogen bonding; then this cluster reacts with the isocyanide in a concerted way to give the cyclic transition state **2.6** involving all three components. Finally, the intramolecular acyl transfer (the so-called Mumm rearrangement) occurs, affording the final Passerini 3CR product **2.7**. Some experimental and theoretical evidences support this mechanism: the reaction takes place efficiently in apolar solvent, and with high concentration of reactants.

Recently, some theoretical studies have been carried out, giving new interesting contributions in the elucidation of Passerini reaction mechanism.

Maeda et al.[21] suggested that an additional carboxylic acid molecule may be involved in the final Mumm rearrangement, forming a bicyclic intermediate (*Scheme 2.5*). This second molecule of carboxylic acid act as a catalyst.



Scheme 2.5 Passerini mechanism proposed by *Maeda*

Using DFT calculations, Ramozzi and al.[22] confirmed the role of a second carboxylic acid molecule as catalysts. Nevertheless, their calculations indicate the nitrilium ion as a relevant intermediate for the Passerini reaction. It is stable in solution, and its formation is the rate-determining step of the process. Consequently, the better efficiency of the Passerini reaction in aprotic solvents is not due to the nonexistence of the nitrilium, as always explained, but rather due to the absence of hydrogen bonds

network with the solvent during the formation of the nitrilium, which would otherwise decrease the reactivity of the reactants. Thus, from these results, they propose a new mechanism pathway for the Passerini reaction (*Scheme 2.6)*.

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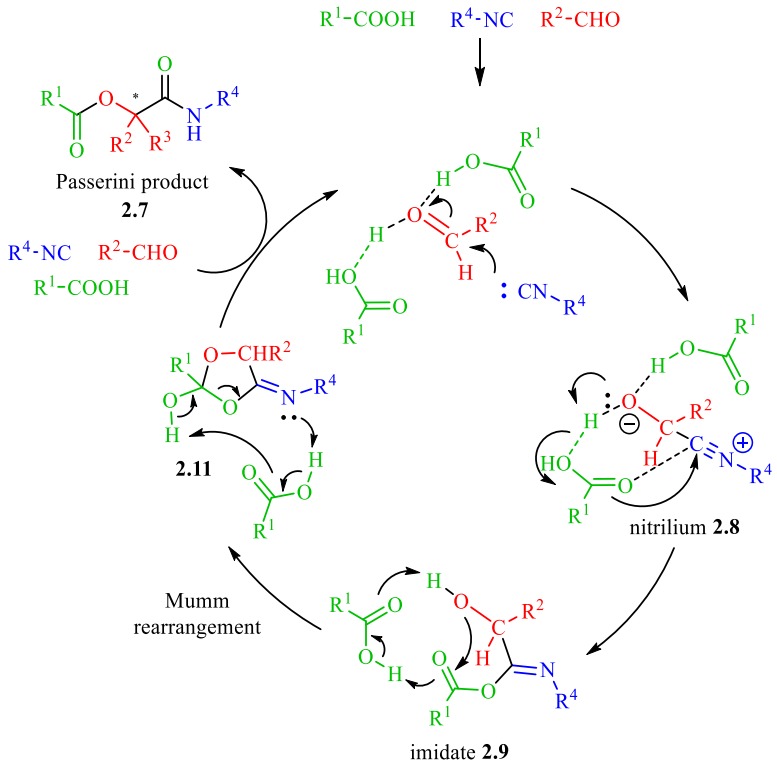


Scheme 2.6 Passerini mechanism proposed by *Ramozzi*

2.2.2.2 Stereocontrol in Passerini reaction

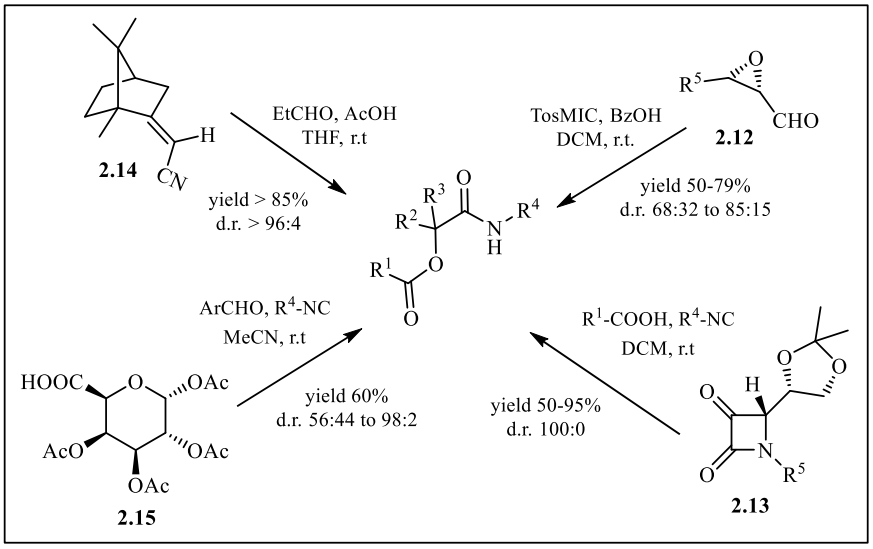
In Passerini reaction, one stereogenic centre is always formed in the process, if the oxo-component is an aldehyde (except for formaldehyde) or a non-symmetric ketone. In the absence of a chiral source in the reaction medium, the resulting product can only turn out to be racemic. As previously described, to obtain chiral products an asymmetric catalytic reaction or a diastereoselective approach can be performed. For our purpose, the second approach will be explored. Considering Passerini reaction mechanism, since all the three reagents are involved in the rate-determining step[23], theoretically asymmetric induction may be achieved when at least one of them is chiral.

The diastereoselectivity induced by chiral carbonyl compounds is usually rather scarce or moderate. This outcome is quite surprising since it is well-known that aldehydes with a stereogenic centre at the α-position are often responsible for good stereochemical control during nucleophilic additions. A possible explanation may be the low stereochemical requirements of the involved nucleophile, *i.e.* the isocyanide. Anyway, some good results are obtained using chiral 2,3-epoxy aldehydes **2.12**, in

presence of tosylmethyl isocyanide (TosMIC) and benzoic acid[24], or chiral lactams **2.13** in the synthesis of β-lactam-triazole hybrids as reported by Alcaide and co-workers[25].

Chiral isocyanides usually react with low stereoselectivity. The only exception so far reported has been the rigid and chiral camphor-derivative **2.14**[26], which can be considered a chiral auxiliary and removed after the condensation reaction to give the corresponding carboxylic acid or ester.

Among the different chiral acids tested for the Passerini reaction, galacturonic derivative **2.15** showed the best level of diastereoselectivity[27].

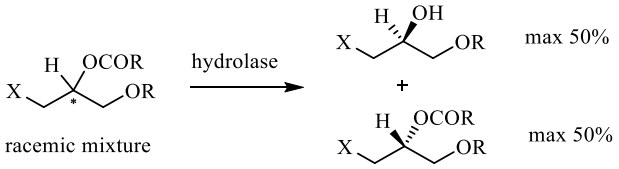


Scheme 2.7 Passerini reaction with chiral substrates

2.3 Enzymatic desymmetrization

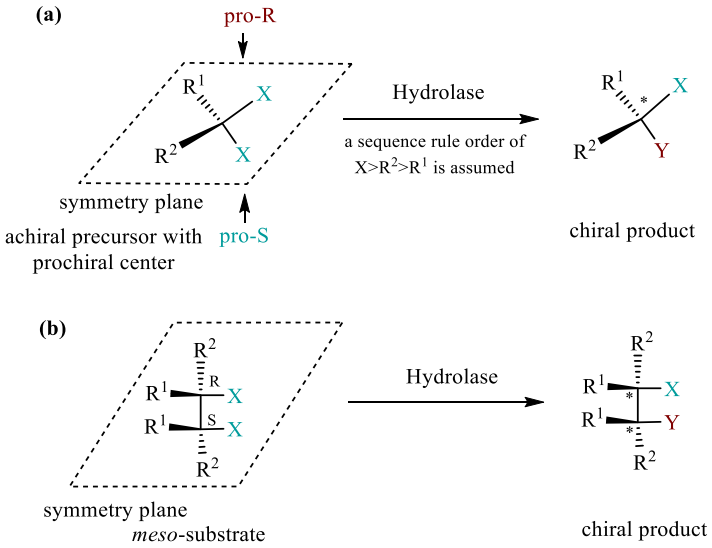
Due to the increasing demand for chiral substrates to conduct diastereoselective multicomponent reactions, biocatalysis represents a fundamental tool in organic synthesis. Different strategies exist to obtain enantiomerically pure compounds. Among them, kinetic resolution and desymmetrization are of particular interest.

In the first approach (*Scheme 2.8*), the substrate is a racemic mixture. The enzyme can differentiate the two enantiomers, converting one of the two at a higher rate. The maximum yield in kinetic resolutions is 50% because the starting material is racemic and this is, of course, the main drawback. To overcome this problem, a method called dynamic kinetic resolution (DKR), have been developed. The aspects of this approach will not be further discussed, as they are not object of this thesis.



Scheme 2.8 A kinetic resolution

The second possibility is to use an achiral substrate (prochiral or *meso* compounds, which are prostereogenic) and to selectively convert it into a single enantiomer. Thus, if the substrate has two chemically identical but enantiotopic groups X and it undergoes the desymmetrization in the presence of an enzyme, a chiral discrimination between these two groups occurs. The transformation of group X into Y is not equivalent for both the groups and a chiral product is obtained (*Scheme 2.9a*). In contrast to a kinetic resolution, the theoretical yield of these conversions is 100%, because there is no more the limitation of the racemic substrate. Similarly, the enzyme can distinguish, the two chemically identical groups X, positioned on carbon atoms of opposite (R, S)-configuration in a *meso* substrates, leading to a single enantiopure compound (*Scheme 2.9b*).

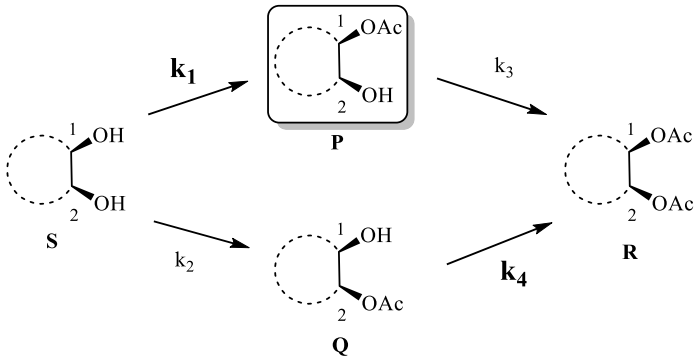


Scheme 2.9 Enzymatic desymmetrization of a) a prochiral and b) of a *meso*-substrate

Therefore, through the desymmetrization process, the symmetry elements that preclude chirality on the substrate are eliminated, thus achieving an enantioselective transformation.

The kinetics of the reactions described above is not so simple (*Scheme 2.10*). A first enantioselective transformation of a prochiral or a *meso* substrate (**S**) leads to the production of two enantiomeric products (**P** and **Q**) at different rates, determined by the rate constants k_1 and k_2, respectively. The monoester resulting from the first

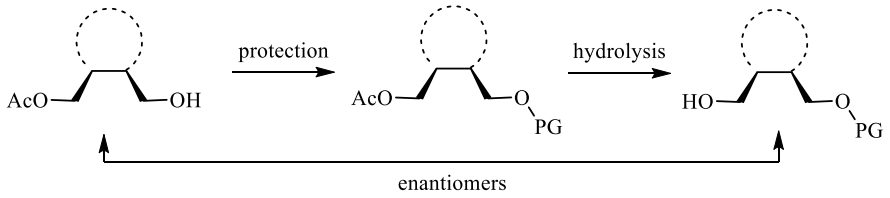
acylation reaction can undergo a second acylation, to yield an achiral diacetate (**R**). However, enzymes usually show preference for the reactive groups with the same chirality. The optical purity of the product depends on four rate constants, k_1 through k_4. Therefore, it is possible to demonstrate that, if $k_1 > k_2$, the second acylation will be carried out on the enzyme's favourite branch and consequently $k_4 > k_3$.



Scheme 2.10 Kinetics

Notably, the optical purity of the monoester becomes a function of the conversion of the reaction. This is because the "undesired" enantiomer deriving from the first acylation (**Q**), is acylated faster than **P** to the achiral diacetate, and is therefore "eliminated" in situ with the second acylation. Consequently, the e.e. of the desired enantiomer (**P**) is improved, reaching higher values at higher conversion, because of the transformation of the other enantiomer to an achiral species. Obviously, if the double-step occurs the yield will decrease. Thus, the reaction has to be stopped at an advantageous conversion in order to achieve an optimal compromise between yields and e.e.

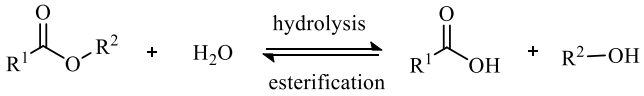
Another advantage of desymmetrization is the possibility to obtain both the enantiomers by performing a hydrolysis reaction of the other acyl group on the compound obtained by the protection of the hydroxyl group. This technique is often referred to as the "*meso*-trick" (*Scheme 2.11*)[28].



Scheme 2.11 Interconversion between two enantiomers toward "*meso*-trick"

Otherwise, both enantiomers can be accessible performing a complementary acylation or hydrolysis reaction, catalysed by the same enzyme. This is possible because of the principle of microscopic reversibility[29], which states that if a certain

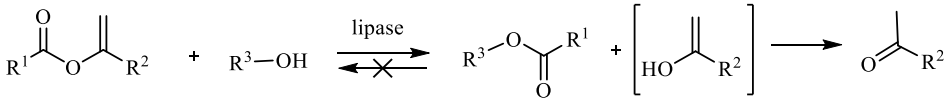
series of steps constitute the mechanism of a forward reaction, the mechanism of the reverse reaction (under the same conditions) is given by the same steps traversed backwards. In the example reported in *Scheme 2.12*, lipase is used to catalyse the "natural" hydrolysis of the ester group and the complementary esterification.



Scheme 2.12 Ester hydrolysis and esterification reaction

The reverse reaction, the esterification, could not be performed in the same conditions of hydrolysis, because the reaction medium is aqueous and, therefore, the equilibrium would be completely shifted towards reagents. However, lipase-catalysed esterification must be performed in an organic solvent and some expedients, such as using anhydrous conditions (molecular sieves or Dean-Stark apparatus) or exploiting an acyl-transfer, have to be used to shift the equilibrium towards the complete ester formation.

In the second case, the reaction performed is not exactly an esterification, but a transesterification (*Scheme 2.13*). Particular species of acyl donors, enol esters (e.g. vinyl ester), are used. These compounds carry an enol as leaving group. Therefore, once they transfer the acyl, an unstable enol is developed, which immediately tautomerizes to the corresponding aldehyde or ketone, thus making the transesterification reaction completely irreversible.



Scheme 2.13 Irreversible enzymatic acylation using enol esters

To conclude this paragraph, enantioselective desymmetrization appears as a powerful tool to rapidly provide complex building blocks bearing several stereogenic centres. Moreover, it is extremely interesting due to the mildness of the conditions and the high selectivity often obtained.

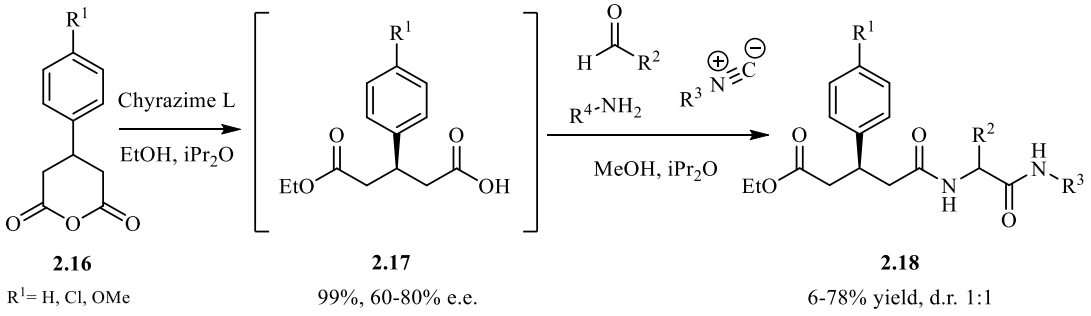
2.4 Applications of Biocatalysis and Multicomponent Reactions

Transesterifications, hydrolysis of esters, and some oxidations are the predominant reactions involved in the desymmetrization of *meso* substrates. These reactions can afford the chiral enantiopure reagents to be used in MCRs to direct the

stereochemical outcome. Some examples given below will show the possibility of coupling desymmetrization reaction with a subsequent IMCR.

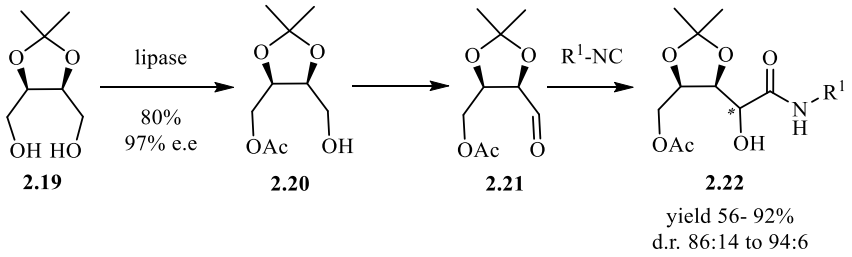
2.4.1 Multicomponent reactions/Enzymatic desymmetrization

The first systematic combination of a MCR and an enzymatic desymmetrization was reported in 2003 by Ostaszewski[30] (*Scheme 2.14*). After generation of enantiomerically enriched glutaric mono esters **2.17** by lipase- mediated desymmetrization, they were submitted to an Ugi 4CR reaction to afford final product **2.18**. Moderate to reasonable e.e. and excellent yields were obtained of desired carboxylic acids. Unfortunately, the Ugi reaction afforded a poor diastereoselection (as is typical if using chiral carboxylic acids[31]). A similar approach was used for the Passerini reaction as well[32].



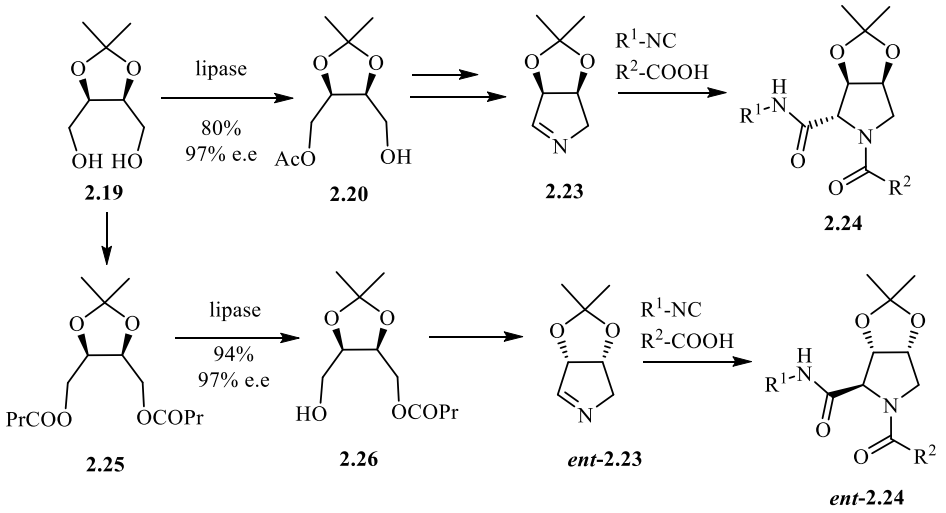
Scheme 2.14 Combination of enzymatic desymmetrization of an anhydride with a subsequent Ugi reaction

Another component for Ugi and Passerini reactions that can be efficiently obtained, although not directly, by lipase-catalysed desymmetrization is the aldehyde. These compounds are not easily obtained directly in enantiopure form through biocatalytic methodologies but can be prepared by oxidation of chiral primary alcohols. An example reported by us[33] involves the desymmetrization of diol **2.19** to afford monoacetate **2.20**, followed by oxidation to the corresponding aldehydes, which is used in diastereoselectve Passerini reactions (*Scheme 2.15*).



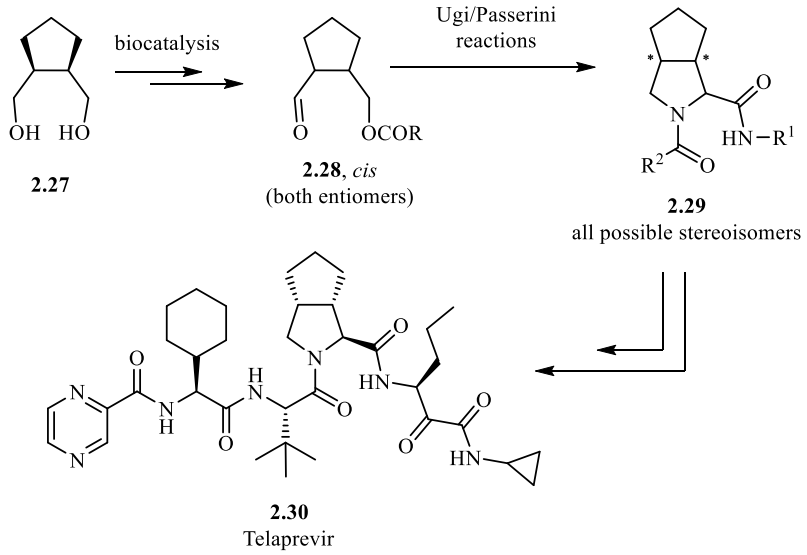
Scheme 2.15 Combination of enzymatic desymmetrization of diol with a subsequent Passerini reaction

Similarly, starting from the same diol **2.19** an enzymatic desymmetrization and subsequent formation of chiral pyrrolines were performed affording the chiral substrates for a diastereoselective Ugi-Joulliè reaction[34] (*Scheme 2.16*). Noteworthy, both enantiomers of the final product are obtained starting from the monoacetate **2.20** and complementary monobutyrate **2.26**.



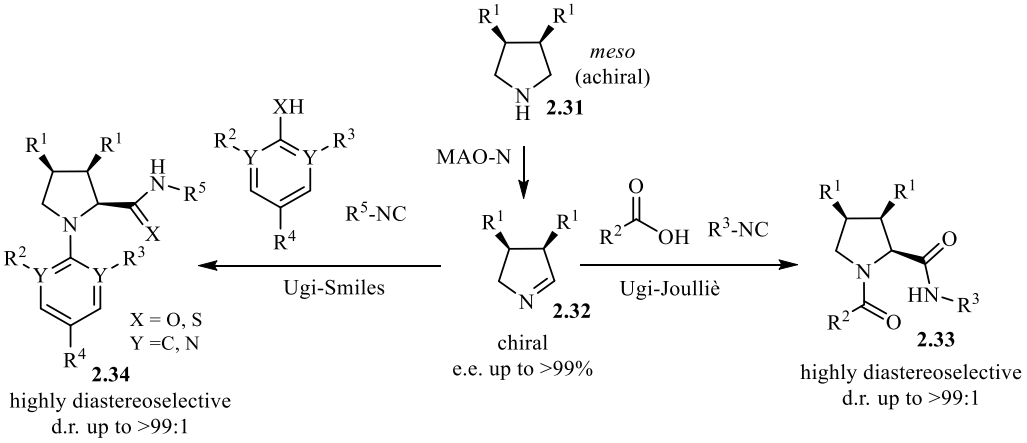
Scheme 2.16 Combination of enzymatic desymmetrization of a diol with a subsequent Ugi-Julliè reaction

Starting from diol **2.27** our group reported another example of coupling biocatalysis and multicomponent reactions. In this case, this strategy was employed for the synthesis of the antiviral drug Telaprevir (*Scheme 2.17*)[35].



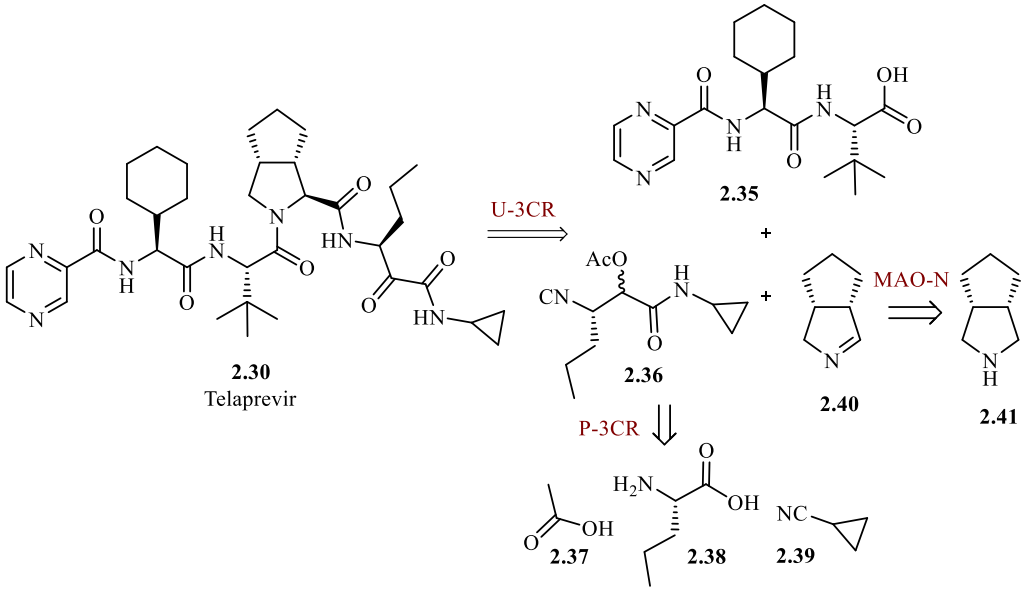
Scheme 2.17 Synthesis of Telaprevir through desymmetrization- IMCRs sequence

Another very useful strategy that combines desymmetrization reaction with IMCRs was recently disclosed by Turner and Orru (*Scheme 2.18*). Optically pure 3,4-disubstituted 1-pyrrolines **2.32** are generated from the corresponding *meso*-pyrrolidines **2.31** by biocatalytic desymmetrization with an engineered MAO-N (monoamine oxidase) enzyme. These compounds are then employed in both an Ugi-Joullièˆ[36] and Ugi-Smiles[37] multicomponent reaction.



Scheme 2.18 General MAO-MCR sequence

According to this strategy, Ruijter *et al*. also developed a short and efficient synthesis of Telaprevir using a biotransformation and two multicomponent reactions as the key steps (*Scheme 2.19*)[38].

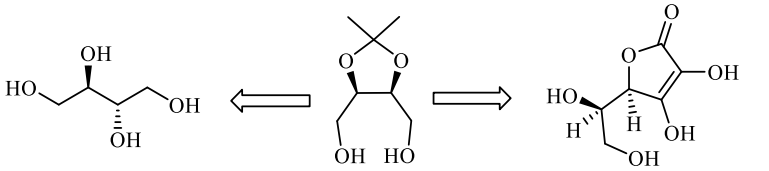


Scheme 2.19 Synthesis of Telaprevir through MAO-MCR sequence

2.5 Integrating biocatalysis and MCRs with renewable sources

As shown above, the combination of biocatalytic principles with MCRs strategies would represent a highly desirable approach towards the green stereoselective synthesis of chiral densely functionalized compounds. Nevertheless, to achieve a real green and sustainable strategy, this is not enough: not only the synthetic methods has to be green, but we need green starting materials as well. Therefore, the development of new green strategies, as the coupling of biocatalysis and MCRs, that can afford complex molecule starting from renewable feedstocks is the new objective for chemists.

Within this context, the research project, I worked on, exploited the coupling of biocatalysis and Passerini multicomponent reaction starting from cyclic *meso*-diol **2.19**. This compound can be efficiently transformed into chiral compounds by desymmetrization reaction affording a useful intermediate for the multicomponent synthesis of complex molecules. In addition, it can be easily obtained from bio-based starting material and, thus, it can be considered a renewable compound. In fact, it is chemically derived from erythritol **2.42** or D-isoascorbic acid **2.43**, both obtainable from biomass (*Scheme 2.20*). The use of these starting materials presents different advantages. In fact, they are renewable feedstock, commercially and easily available, and cheap.

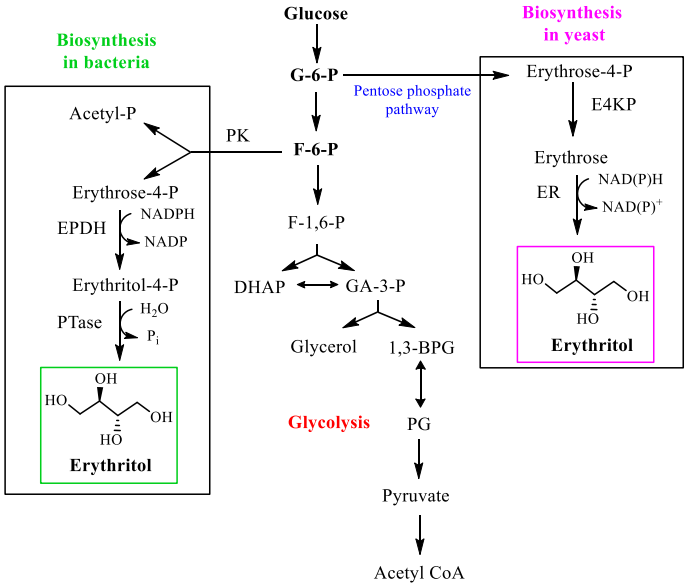


Erythritol **2.42** *meso*-diol **2.19** D-isoascorbic acid **2.43**

Scheme 2.20 Retrosynthetic approach for diol 2.19

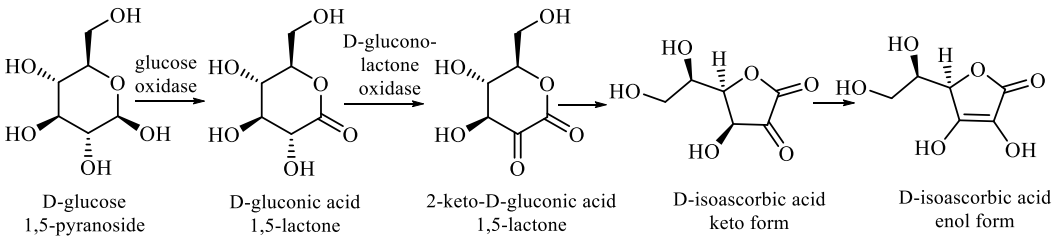
Erythritol is a *meso* natural four-carbon polyol widely distributed in nature, that is considered a promising intermediate building block due to its easily conversion into polyfunctionalized chiral compounds. Erythritol occurs as a metabolite or storage compound in seaweeds and fungi and is also a component of a number of common fruits and fermented food. Its most famous application is undoubtedly as a "non-caloric" sweetener. Osmophilic yeasts and some bacteria can produce it.

The biosynthesis by yeast is a multi-step metabolic pathway, which proceeds mainly via the pentose phosphate pathway, while bacteria produce it as a by-product of NADPH regeneration for phosphoketolase pathway (*Scheme 2.21*). This was exploited for its large-scale production, which uses fermentative processes with pure glucose and sucrose from chemically and enzymatically hydrolysed wheat and corn starches[39].



Scheme 2.21 Biosynthesis of erythritol in yeasts and bacteria

D-isoascorbic acid (also referred as erythorbic acid or D-araboascorbic acid) is an epimer at C5 of ascorbic acid, with similar physicochemical properties. As a vegetable-derived food additive, it can be considered natural. Because of its strong reducing properties, erythorbic acid has similar technologic applications to ascorbic acid as a water-soluble antioxidant[40] and is widely used as an additive in processed foods[41]. D-isoascorbic acid and its salts are considered GRAS (generally recognized as safe) by the US Food and Drug Administration and are approved as food ingredients E315 (free acid) and E316 (sodium salt) in Europe. It is synthesized by Penicillium from D-glucose, D-glucono-γ and β-lactone, D-gluconate, sucrose, maltose, and starch. Glucose and gluconolactones are more suitable substrates than others. The D configuration at C5 allows a short biosynthetic pathway from D-glucose (*Scheme 2.22*).

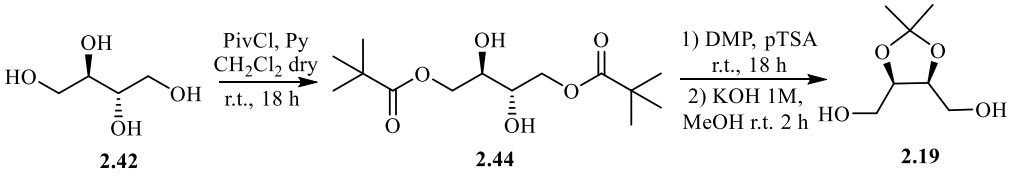


Scheme 2.22 Biosynthesis of D-isoascorbic acid from D-glucose

2.5.1 Synthesis of *meso*-diol from renewable feedstocks

The first part of my project was, therefore, focused on the synthesis of the building block diol **2.19**.

Initially, *meso* diol **2.19** was prepared from commercially available erythritol **2.42**, following a procedure reported in literature[42] and already used in the research group[33-34]. It involves a 3-step sequence, via selective protection of the primary hydroxy groups as pivaloyl esters, protection of the secondary hydroxy groups as acetonide and ester hydrolysis (*Scheme 2.23*).



2.42 **2.44** **2.19**

Scheme 2.23 Synthesis of *meso* diol from erythritol

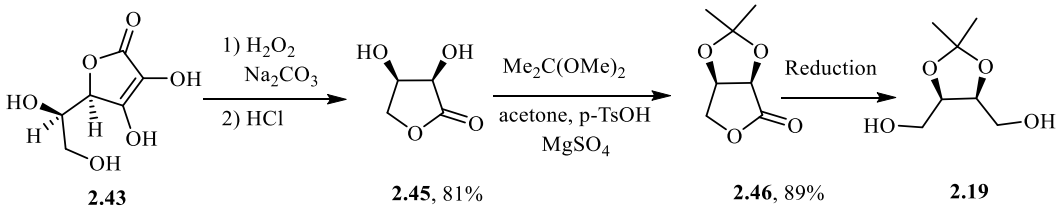
This method presents some critical issues:

- The yield of the first step is rather low (54%) since with the diacylate product **2.44**, other side-products are formed (mono- and tri-acylated compounds).
- Once obtained compound **2.44**, the subsequent protection as acetonide was performed and the crude was directly subjected to the basic hydrolysis. The reaction proceeds with good yield, but the diol is difficult to isolate because of its poor solubility in organic solvents and strong affinity toward water. Therefore, it is not possible to use an extractive work up. Another problem is its sensitivity to acidic environment, which, in some instances, may be responsible for the cleavage of the acetonide group. As a consequence, the control of pH is very important. Furthermore, we observed that the presence of pivalic acid formed during the reaction, if not properly removed, caused the deprotection of the acetonide group. The procedure reported by our group involved the use of solid NH_4Cl; then the crude was filtered, washing with THF/MeOH 9:1 (v/v), and the residue was purified by chromatography. Under these conditions the product **2.19** was obtained with 72% yield. However, the chromatography resulted very troublesome owing to the physical state of the crude: it is a very thick oil difficult to transfer into the column.

Two attempts of improving the work up conditions of the hydrolysis reaction and isolate the product has been accomplished. The first one involved the treatment of the reaction solution with a strongly acid cation exchange resin (Amberlite® IR 120 hydrogen form) until pH 6/7 to neutralize KOH; the other one involved a liquid-liquid continuous extraction. The main disadvantage of the first procedure is that the use of the acid resin can cause the hydrolysis of acetonide and lead to the starting erythritol

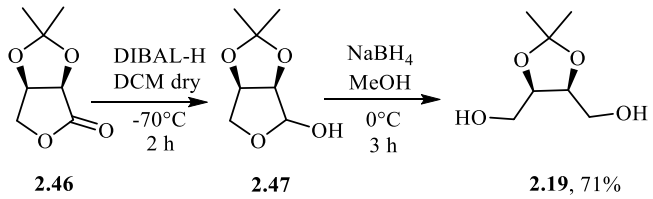
2.42. To overcome this problem, after treatment with the resin, K_2CO_3 was added (until pH = 10). Although the yield obtained with this method was quite satisfactory (71% yield), the purification of the product resulted troublesome since K_2CO_3 was difficult to remove, even by chromatography. Therefore, we tried to carry out a liquid-liquid continuous extraction of the crude. A similar yield (75%) was obtained but this procedure requires a specific equipment and several days -nine in our case- to have a quantitative extraction of the product.

Due to the previous results, another strategy for the synthesis of diol **2.19** starting from D-isoascorbic acid **2.43** was attempted (*Scheme 2.24*). As shown in the scheme, this second approach has the main disadvantage of starting from a chiral substrate, D-isoascorbic acid, to afford an achiral product, the diol, which will be used for the synthesis of chiral building blocks in a later step of the project. On the other hand, this disadvantage is compensated for by the better yields obtained in the various steps.



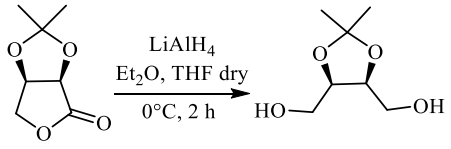
Scheme 2.24 Synthesis of diol from D-isoascorbic acid

The first two steps to obtain compound **2.46** were performed following a procedure reported in literature[43], that involved the oxidation of D-isoascorbic acid to lactone **2.45** in presence of hydrogen peroxide and the subsequent protection with acetonide. The reaction gave the product with a high yield (81%). For the reduction of the lactone **2.45** two different strategies have been compared. The first strategy, reported in literature[44], involves the reduction of the lactone with DIBAL-H to lactol **2.47** and the subsequently reduction with $NaBH_4$ to obtain the diol **2.19** (*Scheme 2.25*).



Scheme 2.25 First strategy in two steps

The second strategy (*Scheme 2.26*) was performed in only one step with $LiAlH_4$.



2.46 **2.19**, 82%

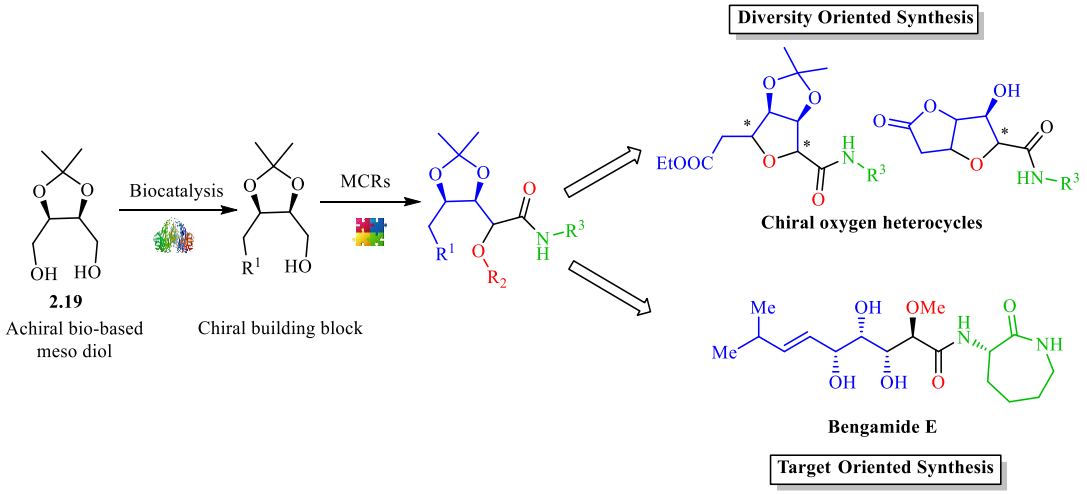
Scheme 2.26 Second strategy in one step

The major advantage of this second procedure is the possibility to avoid the purification of the product, that can directly be used for the next step. Thus, only one purification with chromatography, after the protection as acetonide, is necessary. In addition, this procedure avoids acidic environment that could be responsible for the cleavage of the acetonide group and extractive work up. This is a crucial point since the intermediates and the final diol are slightly soluble in organic solvents and have a strong affinity toward water.

2.6 Conclusion

The union of renewable feedstocks, biocatalysis and MCRs in a single integrated general strategy represents a powerful tool to achieve a sustainable synthesis. Enantioselective desymmetrization strategies for the synthesis of small chiral molecules create added-value building blocks from simple, easily accessible renewable starting materials. MCRs are surely one of the most convenient methods for producing polyfunctionalized complex structures in a highly convergent manner. Despite the great potential of this useful combination few examples have been described in literature whereas more examples of the combination of just two of them can be found.

In this context, we provide an example of the use of this "powerful trio" for the stereoselective synthesis of functionalized complex molecules. Exploiting biocatalysis starting from *meso* diol **2.19**, we afforded chiral precursors, which were conveniently used in a Passerini reaction, to direct the stereochemical outcome of the process. Once obtained in a diastereoselective way the Passerini products, they were submitted to further modification, to access diverse chiral heterocycle systems and to afford a interesting natural product as Bengamide E.



Scheme 2.27 Research plan

2.7 Experimental section

2.7.1 General remarks

NMR spectra were taken at room temperature in $CDCl_3$ or in d_6-DMSO at 300 MHz (^1H), and 75 MHz (^{13}C), using, as internal standard, TMS (^1H-NMR: 0.000 ppm) or the central peak of $CDCl_3$ (^{13}C: 77.160 ppm). Chemical shifts are reported in ppm (δ scale). Coupling constants are reported in Hertz. Resonances are described as s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet) and m (multiplet) or combinations thereof. Peak assignments were made with the aid of gCOSY and gHSQC experiments. In ABX system, the proton A is considered upfield and B downfield.

TLC analyses were carried out on silica gel plates and viewed at UV (254 nm) and developed with Hanessian stain (dipping into a solution of $(NH_4)_4MoO_4 \cdot 4H_2O$ (21 g) and $Ce(SO_4)_2 \cdot 4H_2O$ (1 g) in H_2SO_4 (31 mL) and H_2O (469 mL) and warming).

R_f values were measured after an elution of 7-9 cm.

Column chromatographies were done with the "flash" methodology using 220-400 mesh silica.

All reactions using dry solvents were carried out under a nitrogen atmosphere.

2.7.2 Experimental procedures

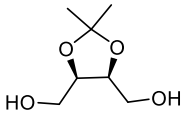
2.7.2.1 Synthesis of meso- diol from erythritol

Compound **2.19** was prepared according to literature procedures, starting from erythritol [42].

White solid, yield 54%.

2.7.2.2 Synthesis of meso- diol from D-(-)-isoascorbic acid

Compounds **2.45** and **2.46** were prepared according to literature procedures, starting from D-isoascorbic acid[43].



((4S,5R)-2,2-dimethyl-1,3-dioxolane-4,5-diyl) dimethanol 2.19

To a suspension of $LiAlH_4$ (1.756 g, 23.14 mmol) in THF dry (45 mL) at 0°C under nitrogen atmosphere, a solution of **2.46** (3.659 g, 23.14 mmol) in dry THF (32 mL) was added dropwise (40 min). The mixture was allowed to reach room temperature and stirred for 3h. Then it was cooled to 0 °C and carefully quenched with Fieser method: deionized water (1.7 mL), NaOH (15%, 1.7 mL) and deionized water (5.1 mL) were sequentially added dropwise. The mixture was stirred until obtaining a white suspension and then filtered through a pad of celite. In order to fully recover the product, which in part remains adsorbed on the aluminates, celite was washed with 900 mL of boiling THF. The filtrate was concentrated, and the residue purified by trituration with Et_2O and then heptane to afford 3.082 g (82% yield) of **2.19** as a white solid. The analytical data conform to those reported in literature[45].

2.8 Bibliography

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CHAPTER 3.
Application of biocatalysis and MCRs to the diversity-oriented synthesis of polyfunctionalized oxygen heterocycles

3.1 Diversity- oriented synthesis (DOS)

The term diversity-oriented synthesis (DOS) first appeared in the chemical literature in 2000 by Stuart Schreiber[1]. DOS aims for the efficient generation of chemical libraries containing structurally diverse and complex molecules, that cover a wide range of the chemical space and have unknown properties. The retrosynthetic analysis does not suite DOS since the target is not known; therefore, the synthetic analysis must be carried out in the forward direction. Synthetic pathways are no longer linear and convergent as in target-oriented synthesis (TOS), but they are rather branched and divergent, and the direction has been switched from simple starting materials to complex and diverse final products. Hence, from a synthetic point of view, desirable features in DOS small molecules libraries are structural diversity and complexity[2].

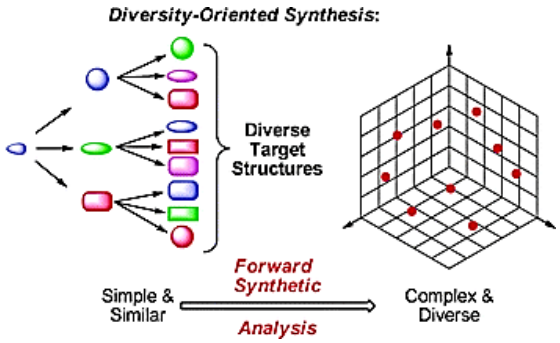
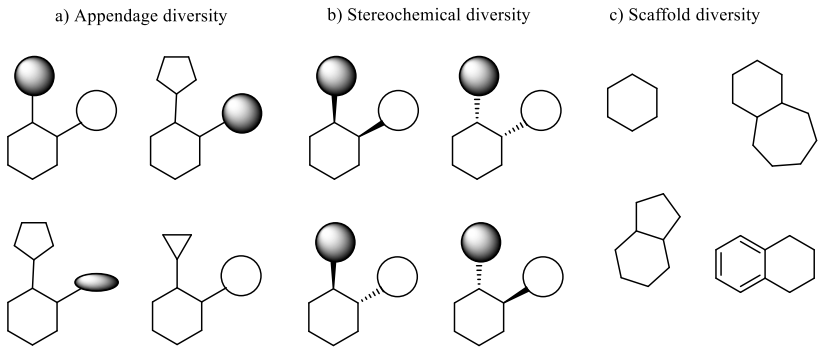


Figure 3.1 Diversity- oriented synthesis.
Taken from *Spandi et al.*[3]

An efficient DOS synthesis must reach the three principal components of structural diversity[4]: a. *appendage or building blocks diversity* that consists in the variation of structural moieties around a common scaffold or of the building blocks used; b. *stereochemical diversity* that is represented by variation in the orientation of functional groups and potential macromolecule-interacting elements; c. *scaffold diversity* that consists in the variation of ring structures and rigid elements resulting in molecules with distinct shapes and scaffolds.

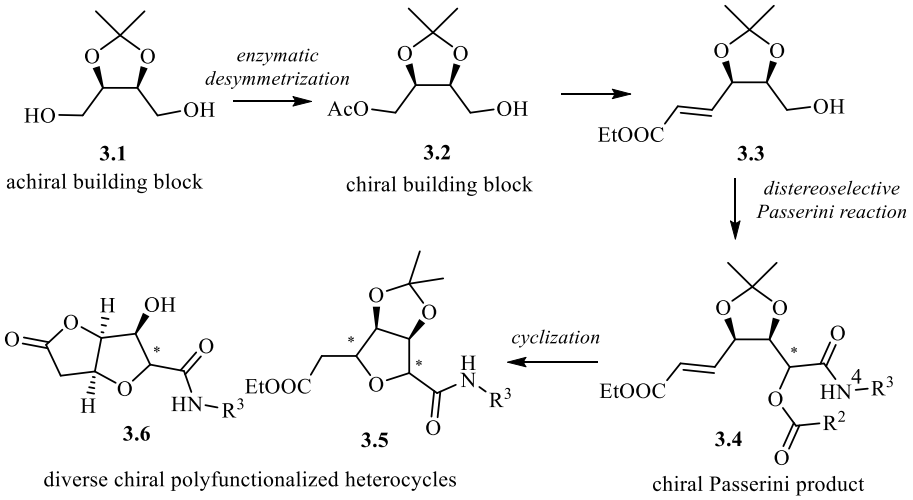


Scheme 3.1 The three fundamental levels of molecular diversity
Taken from *Ruijter et al*[4].

For DOS to succeed, highly efficient versatile, and robust synthetic methodologies are needed. This requires new concepts and novel design approaches. A powerful strategy to address these challenges involves the use of multicomponent reactions, that allow the easily and rapidly generation of complex and diverse small molecules.

One of the main limits of multicomponent reactions is represented by the narrow scaffold diversity that these reactions can achieve. Therefore, this problem can be overcome with two approaches. First, and most difficult, the discovery of novel MCRs. This is not just left to serendipity. On the contrary, in the past years, a rational design approach led to access to new multicomponent processes (this strategy will not be hereby deeper examined). The second approach, commonly referred to as the build/couple/pair strategy[5], involves the combination of already existent MCRs with post-condensation modification approach. The *build phase* involves the synthesis of the building blocks with appropriate chirality and functional groups that can be used for the coupling and subsequent pairing reactions. The second *couple phase* involves intermolecular reactions between the building blocks identified. MCRs play a significant role in this phase to construct the densely functionalized substrate for pair phase. Use of chiral building blocks allows to incorporate all possible stereochemical diversity in the substrate. Finally, in *pair phase*, intramolecular reactions are performed to obtain skeletal diversity. In particular, the most widely employed are cyclization reactions, which allow the obtainment of heterocyclic systems. Among MCRs, Passerini reaction, due to its efficiency and versatility, has been often used as coupling reaction. A wide variety of Passerini post-cyclization reactions is reported in literature[6] to access in only few steps different heterocycles, that could find useful applications in drug discovery as “privileged scaffold”.

In this context, my research project was focused on the synthesis of chiral, bio-based, oxygen heterocycles scaffolds, starting from *meso* diol **3.1**, by coupling biocatalysis, diastereoselective Passerini reaction and post-condensation cyclizations. The general synthetic plan is depicted in *Scheme 3.2*.



Scheme 3.2 Synthetic plan

Diol **3.1** is a symmetrical molecule (*meso* compounds), therefore it is not optically active. To access enantiomerically pure building blocks, it was subjected to lipase-mediated desymmetrization. Then we investigated the manipulation of the enantiomerically pure building block **3.2** in order to introduce appropriate functional groups. With this functionalized molecule available, its use in the Passerini three-component reaction as oxo-compound (with previous oxidation of alcohol functionality) was extensively studied. To achieve a better stereoinduction, a Lewis-acid ($ZnBr_2$) mediated process was performed. As extensively described in *Paragraph 3.2.3*, in fact, previous works demonstrated that the presence of $ZnBr_2$ can enhance the diastereoselection of the Passerini reaction on α-chiral aldehydes[7]. Finally, to broaden the scaffold diversity of this methodology, we studied some cyclization pathways. Some interesting heterocyclic systems were obtained, with control of relative and absolute stereochemistry of the stereogenic centres.

This strategy offers various benefits: the chiral aldehydes are accessible using low-cost and "green" catalysts; both enantiomers are accessible performing a complementary acylation or hydrolysis reaction, catalysed by the same enzyme; diversity can also be achieved by introducing, through functional group manipulations, a variety of alternative appendages that can be exploited for post-MCR cyclizations leading to a variety of non-aromatic heterocyclic scaffolds (exploration of scaffold diversity). These cyclizations may involve not only the CH_2OCOR^2 or NHR^3 groups, but also the double bond as well.

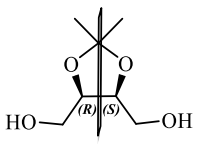
Overall, this synthetic route leads to a fast and sustainable method to access a small library of chiral compounds with a high degree of diversity and complexity and with control of stereochemistry.

3.2 Results and Discussion

The results described in this chapter are organized in three main sections. Firstly, the synthesis of chiral starting material through an enzymatic approach will be discussed (*Paragraph 3.2.1*). Then, the introduction of suitable functional groups will be described (*Paragraph 3.2.2*) and the effect on the diastereoselection in the Passerini reaction will be presented (*Paragraph 3.2.3*). Finally, the transformation of the chiral Passerini adducts previously obtained into some different heterocyclic scaffolds will be shown *(Paragraph 3.2.4*).

3.2.1 Enzymatic synthesis of chiral building block 3.2

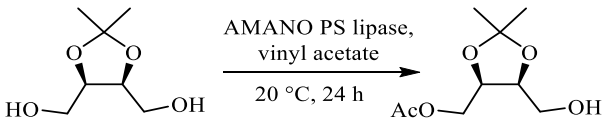
Diol **3.1** is a symmetric molecule, characterized by an internal mirror plane (*Scheme 3.3*). This prevents the molecule to be chiral. To access an enantiomerically pure building block, it was subjected to a lipase- mediated desymmetrization.



3.1

Scheme 3.3 Diol 3.1: a *meso* compound

Due to their chiral active site, lipases are able to discriminate between the two hydroxy groups on the substrate. Thus, only one of the two possible enantiomers can be in principle obtained, with theoretically 100% of both yield and enantiomeric excess (e.e.). The enzymatic reaction was not performed towards the "natural" direction that lipases follow (hydrolysis), but the opposite. Therefore, the reaction is an enzymatic acylation, which converts the diol **3.1** into its corresponding monoester **3.2** (*Scheme 3.4*). This reaction was accomplished following an optimized protocol previously developed by our group[7a] and gave the desired product **3.2** in high yield and e.e.



3.1 **3.2**, 89%, 92% e.e.

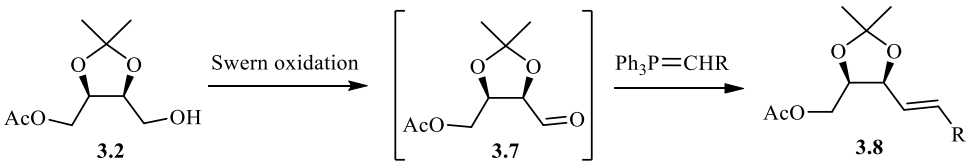
Scheme 3.4 Enzymatic desymmetrization of diol

The enantiomeric excess was determined after benzoylation of the free alcohol, necessary to make the molecule visible to the UV detector, and HPLC analysis with a chiral column.

3.2.2 Functional groups manipulation

The optically active building block can be easily modified to introduce appropriate functional groups that can be used for further synthetic applications after the MCR. Specifically, we decided to introduce a double bond that can be exploited in ring closing metathesis reactions (RCM) or, if conjugated with an ester moiety, it can be used as a Michael acceptor[8].

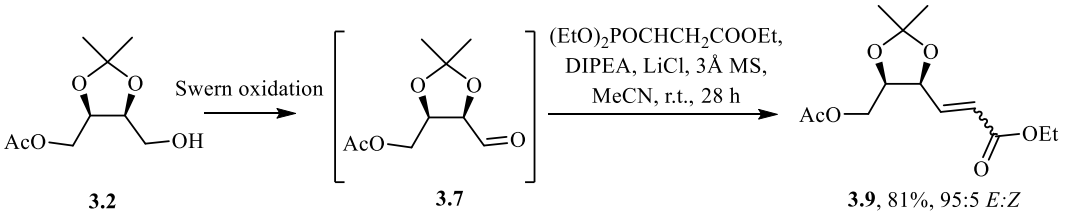
The chiral alcohol was oxidized to the corresponding aldehyde under Swern conditions and the crude was directly subjected to the following olefination. During the olefination reaction an important factor has to be taken into account: preserving the enantiomeric purity obtained through the enzymatic desymmetrization. In fact, in aldehydes with a stereogenic centre in the α-position to the carbonyl function, the α-hydrogen atom shows an enhanced acidity. This gives them the tendency to racemize (in this case epimerize, since the aldehyde has two stereogenic centres), thus leading to the loss of the stereochemical information.



Scheme 3.5 Oxidation and Wittig reaction

Initially, we planned a simple Wittig reaction to introduce a terminal double bond (R = H). The reaction was performed in dry THF at room temperature. For the generation of methylenetriphenylphosphorane, starting from methyltriphenylposphonium bromide, two different bases were employed (LiHMDS and NaHDMS). Unfortunately, no product was obtained. Therefore, the reaction was performed with a stabilized ylide (R = COOEt). The yield of the reaction involving a stabilized ylide was quite good (73%) but the d.r. was not as satisfactory (41:59 *E*: *Z*). Moreover, a partial epimerization in the position α to the carbonyl occurred (about 2%, detected by GC-MS analysis). We also tried to perform the one-pot procedure involving the oxidation with the TEMPO/BAIB system, followed by addition of the Et_3N (for quenching the acetic acid) and the stabilized ylide. In this case, a good yield (89%) and a moderate d.r. (70:30 *E*: *Z*, with desired *E* selectivity) were obtained but, again, a partial epimerization was found (about 6%).

To solve these problems, we decided to perform the Horner-Wadsworth-Emmons reaction (*Scheme 3.6*), using the DIPEA-LiCl system. The use of these reagents is shown to be highly effective for Horner− Wadsworth−Emmons (HWE) olefination of epimerizable aldehydes with phosphonoacetate, affording products with little or no epimerization[9].

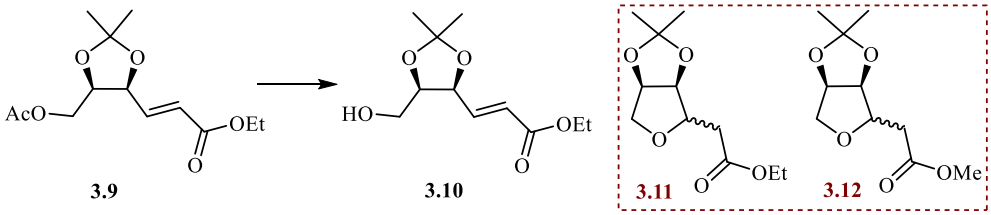


Scheme 3.6 Swern oxidation and Horner-Wadsworth –Emmons reaction

Treatment of the aldehyde with lithium chloride, diisopropylethylamine and triethyl phosphonoacetate furnished the desired product **3.9** with high yield and very good control over the configuration of the double bond (*E: Z*, 95:5, determined by ^{1}H-NMR analysis). The two diastereoisomers can be easily separated by chromatography. Moreover, as expected, no epimerization was observed.

To perform the multicomponent reaction, the acetyl protecting group has to be removed and the chiral alcohol has to be converted into the corresponding aldehyde. The deprotection was carried out on the *E* diastereoisomer and both enzymatic and chemical methods were investigated (*Tables 3.1*).

Table 3.1 Optimization of deprotection conditions of the Ac group

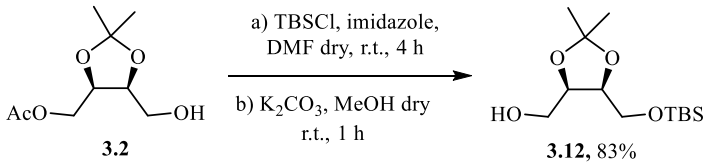


Entry	*Reagent*	*Solvent*	*T*	*Time*	*Product*	*Yield*
1[b]	KOH 1M	MeOH 0.46 M	r.t.	3 h	**3.12**	79%
2[a]		Et_3N: H_2O:EtOH 1:1:5	r.t	6 d	**3.11**	61%
3[b]		Et_3N:H_2O:MeOH 1:1:5	r.t.	30 h	**3.11, 3.12**	30%, 58%
4[c]	CAL-B[h]	buffer[e]: THF 82:18, 0.15 M	r.t.	7 d	**3.10**	54%
5[d]	CAL-B[h]	buffer[e]: THF 82:18, 0.07 M	r.t.	8 d	**3.10**	45%
6[d]	S-PPL[i]	buffer[e]: THF 82:18, 0.07 M	r.t.	14 d	-	
7[c]	CAL-B[h]	buffer[e] THF 82:18 0.07 M	60°C	14 d	-	
8[c]	CAL-B[h]	buffer[e]: iPr_2O 85:15, 0.1 M	60°C	5 d	-	
9[c]	AMANO PS[f]	buffer[e]: iPr_2O 85:15, 0.1 M	60°C	6 d	**3.11**	54%
10[d]	PPL[g]	buffer[e]: iPr_2O 85:15, 0.1 M	r.t.	11 d	-	

[a] d.r. 74:26 anti: syn determined by GC-MS, [b] d.r. not determined, [c] Reaction performed with magnetic stirring or [d] with mechanic stirring, [e] Phosphate buffer at pH 7, [f] AMANO PS: Lipase from *Pseudomonas cepacia*, [g] PPL: Lipase from Porcine pancreas. [h] CAL-B: Lipase from *Candida antarctica,* [I] *S-PPL:* Lipase from Porcine pancreas immobilized on celite,

Unfortunately, neither of them was successful. The desired product **3.10** was obtained in low yield (*Entries 4 and 5*) meanwhile, in most of the cases under the mild basic conditions, tetrahydrofuran **3.11** was always obtained. This is due to the fact that the hydrolysis of the acetate was immediately followed by a fast intramolecular Michael addition of the hydroxy group to the double bond. Moreover, in those examples where the hydrolysis was performed in MeOH as solvent, also a partial or complete transesterification occurred affording **3.12**. The cyclization, even if not optimized, showed a moderate stereoselectivity (74:26). It is noteworthy that, also using lipase-catalysed hydrolysis which works under neutral conditions, in some cases, we isolated considerable amounts of **3.11** as well (*Entry 9*).

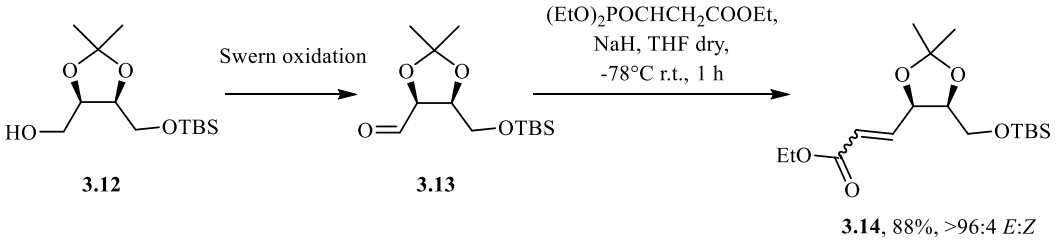
Due to these results, we decided to change the acetyl protecting group with a silyl ether group (TBS: *tert*-butyldimethylsilyl ether). It can be removed under acidic conditions, which should disfavour the Michael reaction. This group can be introduced into the monoacetate through a protection-deprotection sequence (*Scheme 3.7).*



Scheme 3.7 Change of protecting group

In this way, the oxidation reaction will be performed on the other CH_2OH group but, however, the opposite enantiomer can be easily accessed by monohydrolysis of the corresponding diester. In detail, in a previous work conducted by us [7a], the best results were obtained performing the reaction on the dibutyrate with AMANO PS Lipase at 0°C.

The chiral alcohol **3.12** was then submitted to the oxidation-olefination reaction sequence. To improve the yield of the Horner-Wadsworth-Emmons reaction, a different procedure was employed[10]. In particular, we switched to NaH as base and did not use LiCl (*Schema 3.8*). The reaction affords the α,β-unsaturated ester **3.14** in good yield and a high d.r. (*E: Z*, > 96: 4). Epimerization was not observed either.



Scheme 3.8 Optimized Horner-Wadsworth-Emmons reaction

Removal of the silyl ether group was not as simple as we expected. Classical deprotection conditions involve treatment with tetra-n-butylammonium fluoride but, again, most likely due to the slightly basic conditions, we obtained as expected the heterocycle derived from Michael addition. On the other hand, under acidic conditions, we observed the cleavage also of the acetonide group (as reported in *Table 3.2* with HF 40%[7a] and H_2SiF_6[11]). Therefore, we tried different conditions[12], requiring the use of HF·pyridinium complex in the presence of pyridine. The initial results were encouraging but we had to perform a careful optimization, in order to optimize the ratio of HF·Py complex vs. pyridine. In addition, we also studied the possibility to use a lower amount of reagent. In fact, based on the procedure reported in literature, a large excess of HF·Py complex was required (125 eq) (*Entry 4, Table 3.2*). We decided to keep unchanged the HF·Py complex: pyridine ratio and the concentration of HF and we tried to change the amount of HF·Py complex and the concentration of the substrate. Thus, the reaction was performed using 25 eq of complex and 0.02 M concentration. It proceeds with good yield, but it requires a longer time (*Entry 5, Table 3.2*).

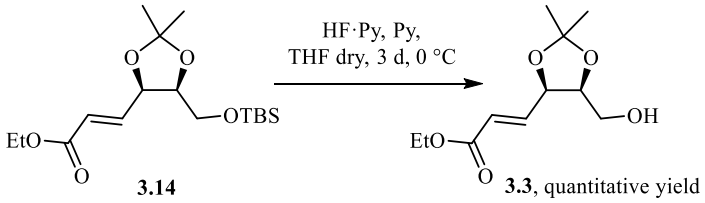
Table 3.2 Optimization of deprotection conditions of the TBS group

Entry	*Reagent*	*Solvent*	*T*	*Time*	*Yield*
1	HF 40%	MeCN 0.023 M	-10°C→r.t.	4 h	-
2	H_2SiF_6 (0.042 eq) Et_3N (0.17 eq)	MeCN 0.1 M	0°C	3 h	30%
3	HF·Py (125 eq)	THF 0.04 M	0°C	4 h	71%
4	HF·Py (125 eq) Py (1.3 eq[a])	THF 0.04 M[b], 5 M[c]	0°C	30 h	Quantitative
5	HF·Py (25 eq) Py (1.3 eq[a])	THF 0.2 M[b], 5 M[c]	0°C	4 d	93%
6	HF·Py (35 eq) Py (1.3 eq[a])	THF 0.145 M[b], 5 M[c]	0°C	3 d	Quantitative

[a] Respect to HF, [b] concentration of the substrate, [d] concentration of HF

An optimal compromise was found using 35 eq. of HF·Py complex with a concentration of the substrate of 0.145 M (*Entry 6, Table 3.2)*. In these conditions, the

desired product **3.14** was obtained in only three days with high yield, employing less equivalent than the initial procedure (*Scheme 3.9*).



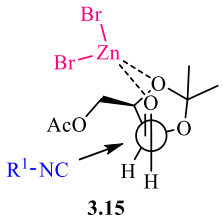
Scheme 3.9 Deprotection of TBS group

3.2.3 Oxidation and diastereoselective Passerini reaction

Compound **3.3** is the precursor of an aldehyde that can be used as carbonyl input in a diastereoselective multicomponent reaction.

Generally, chiral aldehydes with a stereogenic centre in the α-position have been rarely used in MCRs. However, during two projects[7], our group have gained some experience on the behaviour in Passerini reactions of some α-chiral aldehydes having two contiguous stereogenic centres (such as our aldehyde) derived from enzymatic desymmetrization of *meso* compounds. In this case, the Passerini reaction is highly conservative even with branched aldehydes with or without the presence of a heteroatom in α-position. Besides, these studies show that the presence of a Lewis acid can enhance the diastereoselection of the reaction.

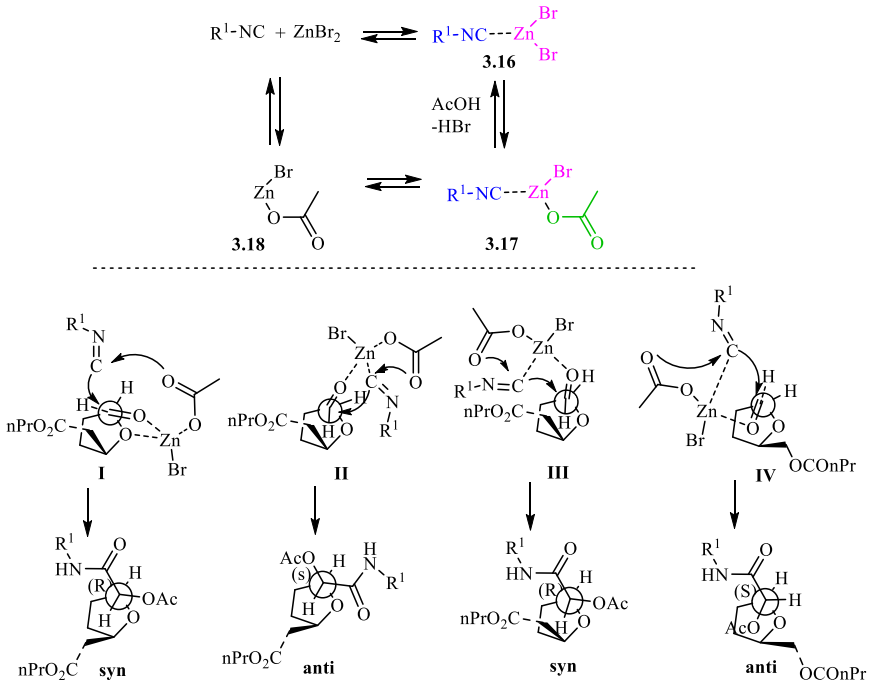
Initially, this fact was explained by the possibility of chelation realized by Lewis acid with the aldehyde oxygen and one of dioxolane oxygens that lead to a 6-membered chelate **3.15**. A contemporary activation of the carbonyl compound to the nucleophilic addition of the isocyanide and the formation of a more rigid transition state is realized. In this hypothesis the attack of the *Si* face of the carbonyl is sterically less hindered and, consequently, the *anti* product is obtained (*Scheme 3.10*).



Scheme 3.10 Rigid transition state with Lewis acid coordination

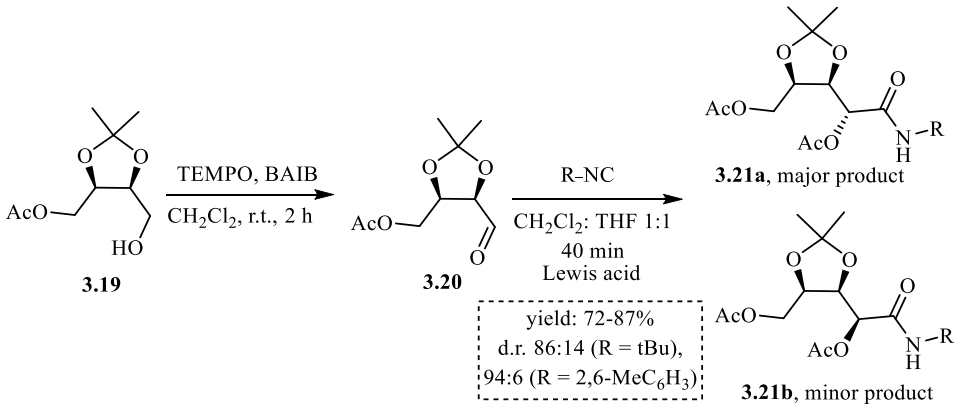
Recently, this hypothesis has been revised by the group, on the base of new experimental results[13] obtained on an aldehyde derived from hydroxymethyl furfural (HMF). Based on the initial hypothesis (chelation between zinc and aldehyde), the

slow addition of the isocyanide to the premixed mixture of aldehyde and $ZnBr_2$ should have led to an improvement in diastereoselection. Oppositely, a poor diastereoselection was observed, meanwhile good results were obtained by the slow addition of the aldehyde and carboxylic acid to the pre-mixed mixture of isocyanide and $ZnBr_2$. This can be explained with the formation of a complex between the isocyanide and $ZnBr_2$ **3.16**, that, through an exchange reaction with acetic acid, can lead to compound **3.17**, the real reacting species. The chelated transition state **I**, initially hypothesized, would favour the *syn* product due to the steric encumbrance of the lower carbonyl face. Thus, it is more reasonable to assume the chelation of the aldehyde and complex **3.17** as shown in transition states **II-IV**. Then, the steric strain is expected to favour **II**, leading to the *anti* isomer. The same transition state is likely to be favoured under "normal" Passerini conditions, but the increased selectivity with the zinc modifications may be due to the more rigid transition states and the higher steric demand of the carboxylate group.



Scheme 3.11 Recent hypothesis for diastereoselection mechanism

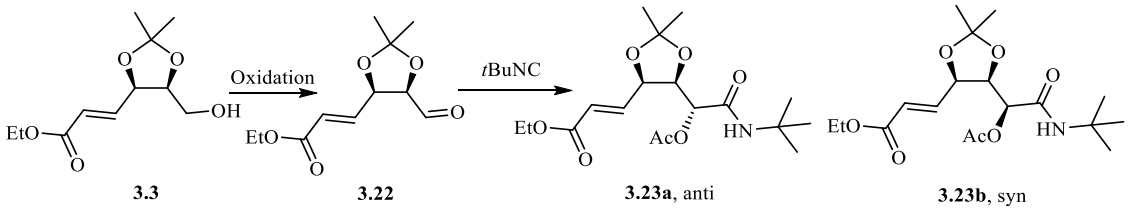
An optimization of oxidation and Passerini reaction on aldehydes derived from erythritol was performed by the group[7b]. The final conditions are reported in *Scheme 3.12* and gave product **3.21** in high yield and d.r.



Scheme 3.12 One-pot oxidation-Passerini reaction

Starting from these results we decided to employ our aldehyde derived from alcohol **3.3** to perform the multicomponent reaction. For our purpose, we had first to oxidize the alcohol to the aldehyde **3.22** and then we decided to study the Passerini reaction using acetic acid and *t*-BuNC.

Table 3.3 Optimization of oxidation and Passerini reaction

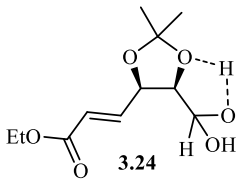


Entry	*Oxidation*[a]	*Time*	*Passerini reaction*[b]	*Time*	*Solvent*	*Yield*	*d.r.*[c] *a:b*
1	TEMPO BAIB	2 h	*t*BuNC	3 h	DCM	64%	61:39
2[d]	TEMPO BAIB	3 h	*t*BuNC	1 h	DCM	56%	71:29
3	Swern	1 h	*t*BuNC, AcOH	1.30 h	DCM	92%	59:41
4	Swern	1 h	*t*BuNC, AcOH	31 h	THF	97%	51:49
5	Swern	1 h	*t*BuNC, AcOH $ZnBr_2$	4 h	THF	78%	77:23

[a] Both procedures for oxidation were performed in dry DCM**,** [b] Passerini reaction was performed with 1.1 eq of tBuNC and AcOH and 0.4 eq of $ZnBr_2$ at 20°C under N_2 atmosphere, [c] determined by ^{1}H-NMR analysis on the crude, [d] the reactions were performed adding 3Å molecular sieves.

The configuration of the newly formed stereogenic centre of the Passerini reaction products was determined at a later stage of the study, by ^{1}H-NMR analysis on a cyclic derivative of them (*Paragraph 3.2.4.2*).

We initially utilized the conditions previously optimized involving the one-pot procedure with the TEMPO/BAIB (bis-acetoxyiodosobenzene) system oxidation, followed by addition of the isocyanide[7b]. The TEMPO/BAIB protocol represents a good example of waste recycling in a tandem procedure; in fact, it allows the oxidation of primary alcohol to the corresponding aldehyde and develops acetic acid, which can be exploited as acidic component for the Passerini reaction. Therefore, during the oxidation step two out of three components of the Passerini reaction are produced (*i.e.* the aldehyde and the acid) and then only the isocyanide needs to be added. The oxidation, however, was not as straightforward as we expected (*Entry 1, Table 3.3*). The main problem we encountered was the overoxidation of the aldehyde to the corresponding carboxylic acid, which of course competed with acetic acid in the Passerini reaction, lowering the overall yield. The overoxidation is favoured by the formation of the hydrated form of the aldehyde (*Scheme 3.13*), likely stabilized by the inductive effect of the α-oxygen atom.



3.24

Scheme 3.13 Hydrated form of aldehyde

Therefore, we tried to suppress this unwanted reaction working under strictly anhydrous conditions (molecular sieves). As shown in *Table 4, Entry 2* a shorter reaction time was observed and, even if the yield decreased, there was a slightly improvement on diastereoisomeric ratio. However, the result was not very satisfactory.

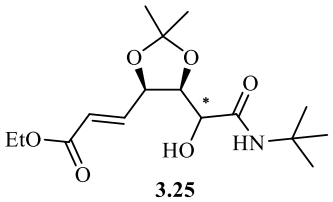
To overcome this problem the Swern oxidation was employed. Even if Swern oxidation eliminates the one-pot procedure, this approach avoids overoxidation of aldehyde. Furthermore, this oxidation method allows to use any carboxylic acid as input in the Passerini reaction and, contrary to one-pot methodologies, allows to change solvent and other reaction conditions of the Passerini reaction.

A brief optimization of the work up conditions for the Swern reaction was performed. In particular, the reaction was extracted with Et_2O, $(NH_4)H_2PO_4$ and 1 M HCl, making sure of the acidity of the aqueous phase (pH 4/5), to remove all the base before evaporation. Then the crude aldehyde was thoroughly dried by azeotropic water removal with toluene and kept under vacuum for one hour. As the aldehyde is not stable on silica, we decided to avoid the chromatographic purification; so, after the workup, the crude product was directly employed in the multicomponent step.

Since the oxidation step was optimized, we decided to improve the diastereoisomeric ratio of the Passerini reaction; indeed, as reported in *Entry 3, Table 3.3*, avoiding the use of a Lewis acid the diastereoselctivity is quite low.

Due to the low solubility of $ZnBr_2$ in DCM, we decide to use THF as solvent, which is able to completely dissolve the $ZnBr_2$. Initially, we performed the reaction without the Lewis acid, obtaining comparable results in terms of yield and d.r. (*Entry 4, Table 3.3*). However, the reaction requires more time (it was complete only after 31 hours) and consequently, a low amount of epimerized aldehyde was observed by GC-MS analysis. Then the reaction with $ZnBr_2$ was performed. We observed that the presence of a catalytic amount of zinc bromide can improve the d.r. but at the same time it decreases the yield (*Entry 5, Table 3.3*).

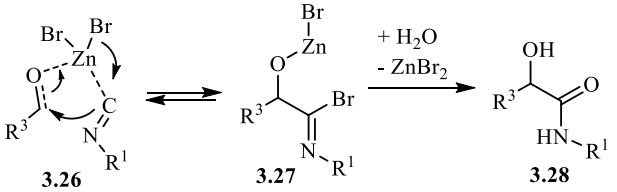
Another problem was the difficulty in determining the d.r. by ^{1}H-NMR analysis, since the presence of the "so-called" truncated Passerini reaction products (compound **3.25**, *Scheme 3.14*) interferes with the integration of the diagnostic peaks. Moreover, the two diastereoisomers could not be separated by HPLC analysis.



3.25

Scheme 3.14 Truncated Passerini product

Since the side-products are not separable by chromatography, we could acetylate the crude product in order to solve this problem. However, experimental data show that the formation of the truncated Passerini product occurs without stereoselection (d.r. about 1:1) indicating that they did not derive from a hydrolysis of **3.23**, but, instead, from an independent process. In particular, the formation of these side-products is promoted by $ZnBr_2$[14], through the generation of intermediate **3.27** without incorporation of carboxylic acid, as shown in *Scheme 3.15*. Therefore, in this case, $ZnBr_2$ doesn't affect the stereoselection of the process.

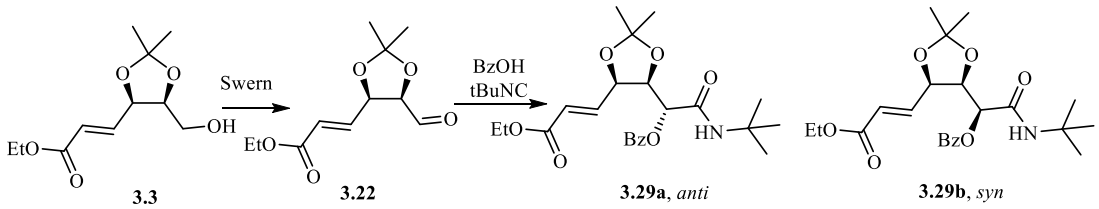


3.26 **3.27** **3.28**

Scheme 3.15 Generation of truncated Passerini product in presence of Lewis acid

This, of course, may affect the overall d.r., since a moderate stereoselectivity is observed for the normal Passerini products. Thus, it was important to suppress the undesired side-reaction.

The difficulty in determine the d.r. was solved using a different carboxylic acid, benzoic acid (BzOH) (*Scheme 3.16*). In fact, the two Passerini products obtained could be separated by HPLC and by column chromatography. Therefore, the d.r. was determined by HPLC analysis and confirmed by ^{1}H-NMR analysis. Moreover, using benzoic acid, the formation of the truncated Passerini products was not observed.



Scheme 3.16 Swern oxidation and Passerini reaction with BzOH

For comparison, the product was first prepared using the standard Passerini conditions (reaction in THF at 20°C without any Lewis acid). Also, with benzoic acid, although the overall yield was good (97%), diastereoselectivity was only poor (d.r. 45:55 *anti*: *syn*). To increase the diasteroselection, the reaction with $ZnBr_2$ was performed. The product was obtained after 17 h with a lower yield (59%) but improved diastereoselection (74: 26 *anti: syn*).

In the attempt of improving the stereocontrol in the Passerini reaction, further optimization was conducted. In particular, temperature effects and the use of zinc carboxylates were investigated (*Table 3.4*). A previous study conducted by our group[13] showed that, with aldehydes containing heteroatoms, the use of zinc carboxylates instead of $ZnBr_2$ could be beneficial in term of d.r. and, at the same time, suppress the formation of truncated Passerini products. To obtain good results, the use of a stoichiometric amount of zinc carboxylates was observed to be necessary, probably because of their lower solubility and milder Lewis acid character. In fact, using a catalytic amount of $Zn(OAc)_2$ and, therefore a stoichiometric amount of acetic acid, the reaction had resulted much faster but a lower d.r. had been detected, suggesting that the normal Passerini mechanism competed significantly with the zinc-mediated pathway.

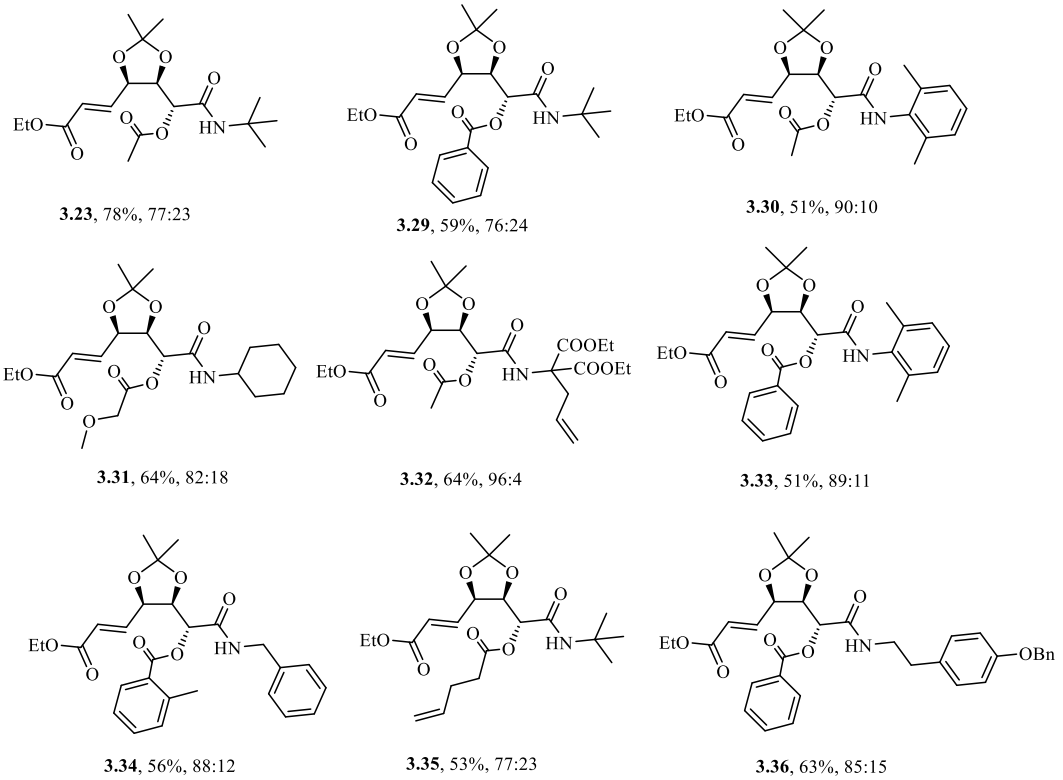
Table 3.4 Optimization of diastereoselectivity

Entry	*Reagents*[a]	*Time*	*Solvent*	*Temperature*	*Yield*	*dr (a:b)*
1	tBuNC $Zn(OAc)_2$	19h	THF	20°C	79%	56:44[b]
2	tBuNC $Zn(OBz)_2$	5h	THF	20°C	36%	64:36[c]
3	tBuNC, BzOH $ZnBr_2$	6d	THF	.- 30°C	39%	65:35[c]
4	tBuNC, BzOH $ZnBr_2$	16h	THF	0°C.	54%	74:26[c]

[a]Passerini reaction was performed with 1.1 eq of tBuNC and BzOH, 0.4 eq of $ZnBr_2$ and 1.1 eq of zinc carboxylate under N_2 atmosphere, [b] determined by ^{1}H-NMR analysis on the crude, [c] determined by HPLC analysis on the crude.

The results obtained showed a negative influence of zinc carboxylates in terms of yield and d.r.. This is probably due to the fact that, since it is not possible to carry out an acidic work up because of the lability of the compound, part of the product remains chelated to the zinc carboxylates. In addition, the lower temperature did not significantly affect the diastereoselection of the process.

Finally, exploiting the optimized conditions (20°C in dry THF with carboxylic acid (1.1 eq), isocyanide (1.1 eq) and $ZnBr_2$ (0.4 eq)), a small library of Passerini adducts was synthesized. Different isocyanides, including aliphatic, aromatic, branched aliphatic and carboxylic acids, including aliphatic and aromatic ones, have been combined with the chiral aldehyde **3.22**. The results obtained were mostly satisfying, as can be seen from *Scheme 3.17* (the major diastereoisomers, *anti*, are shown).



Scheme 3.17 Scope of Passerini reaction

The diastereoselective ratio (determined by ^{1}H-NMR analysis from compound **3.23** to **3.32** and by HPLC from compound **3.33** to **3.36**) was in all cases good, and even higher than for the first model compound, ranging from 77:23 to 96:4. The separation step of the isomers was not always easy to perform but, in every case, it was possible to isolate the major final product as a single isomer. The yields are calculated on two steps on both the diastereoisomers, *anti* and *syn*. For compounds **3.30** and **3.32**, also the presence of truncated Passerini products was detected (about 50% by HPLC analysis on the crude). In order to remove the side-products, the acetylation of the crude product was performed with Ac_2O, Et_3N, and DMAP.

3.2.4 Post-condensation modification

The synthetic possibilities of IMCRs can be increased by all the procedures known as post-condensation transformations. Indeed, considering the high compatibility of MCRs with a variety of unprotected orthogonal functional groups, the scaffold diversity can be greatly enhanced employing suitable functionalized starting materials, which produce secondary modifications spontaneously, in a domino process, or upon treatment with further reagents.

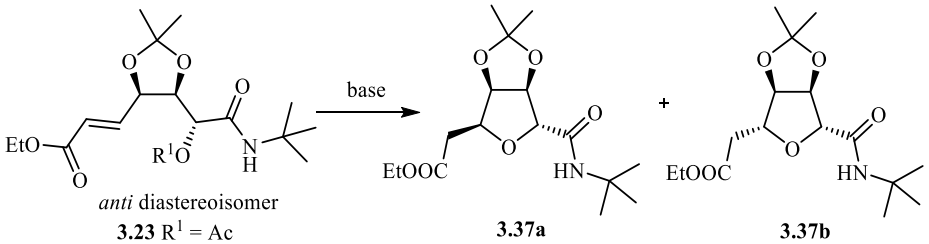
Considering this fact, the cyclization of the erythritol-derived Passerini products was studied quite extensively. Three diverse cyclizations were performed and two different chiral heterocyclic compounds were obtained. From these studies, it was

also possible to assign the absolute stereochemistry of the stereogenic centre formed during the Passerini reaction (*Paragraph 3.2.4.2*).

3.2.4.1 Intramolecular Michael addition

Once we obtained the chiral Passerini adducts, we studied their conversion (as pure diastereomers) into tetrahydrofurans, exploiting an intramolecular Michael addition. To do this, we take advantage of the presence of an alcohol (obtained by hydrolysis) and the α-β, unsaturated ester moiety. The synthetic sequence was carried out separately on both diastereoisomers of Passerini adducts **3.23** and **3.29** and three different hydrolysis conditions were explored.

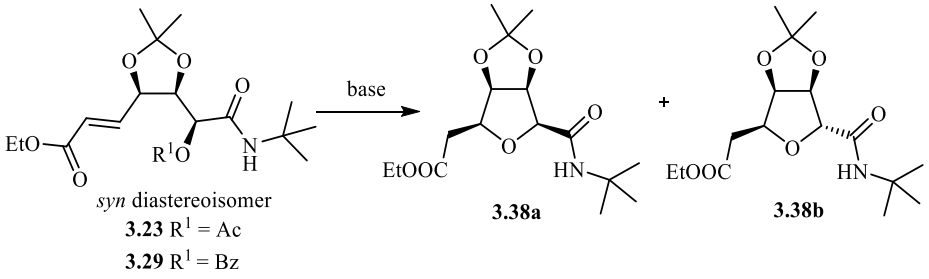
Table 3.5 Intramolecular Michael addition on *anti* diastereoisomer



Entry	*Starting material*	*Reagents*	*Time*	*T*	*Solvent*	*Yield*	*d.r[a] (3.37a:3.37b)*
1	**3.23**	KOH 1M	2h	r.t.	EtOH	33%	91:9
2	**3.23**	EtONa[b] 0.1M	6h	r.t.	EtOH	58%	90:10
3	**3.29**	EtONa[b] 0.1M	5h	r.t.	EtOH	73%	92:8
4	**3.29**	EtOH: Et_3N: H_2O 5:1:1	36h	r.t- 60°C	EtOH	74%	82:18

[a] Determined by ^{1}H-NMR analysis on the crude.
[b] Prepared in situ from Na and EtOH.

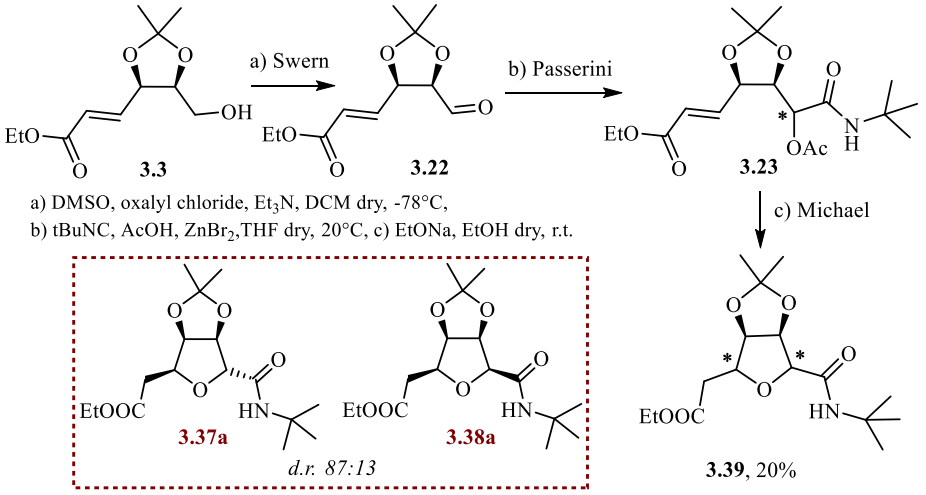
Table 3.6 Intramolecular Michael addition on *syn* diastereoisomer



Entry	*Starting material*	*Reagents*	*Time*	*T*	*Solvent*	*Yield*	*d.r.[a] (3.38a:3.38b)*
1	**3.23**	KOH 1M	2h	r.t.	EtOH	16%	84:16
2	**3.23**	EtONa[b] 0.1M	5h	20°C	EtOH	73%	83:17
3	**3.29**	EtONa[b] 0.1M	22h	0°C	EtOH	83%	89:11
4	**3.29**	EtONa[b] 0.1M	6h	r.t.	EtOH	65%	83:17

The results reported in *Table 3.5* and *Table 3.6* show that the reaction proceeds on both diastereoisomers of Passerini products with good yields and d.r. In particular, the experimental data suggest that the configuration and d.r. of the newly formed stereocentre mostly depend on the configuration of the dimethyldioxolane ring and not on the configuration at C_α, preferably leading to the same stereocentre during the cyclization. After a little optimization, we found the best conditions in the use of EtONa as base in dry EtOH at room temperature

We also tried to perform the cyclization with Swern and Passerini reactions without purification of intermediates. In this way, cyclization was performed on the mixture of the two diastereoisomers of the Passerini product.



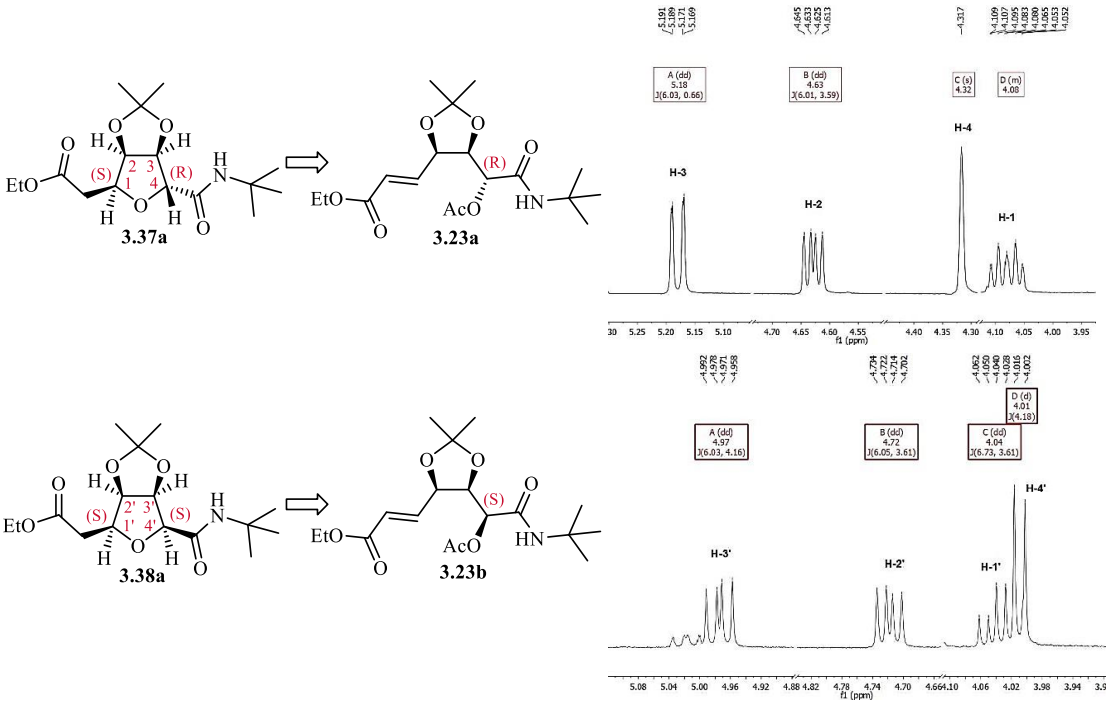
Scheme 3.18 Swern oxidation, Passerini reaction and Michael addition

The final product was obtained with 20% yield and a ratio between the two major diastereoisomers **3.37a:3.38a** of 87:13.

3.2.4.2 Determination of the relative configuration of Passerini products

The stereochemistry of the Passerini products was established after conversion into tetrahydrofuran. These heterocycles are rather rigid compounds and, therefore, this allowed us to unambiguously assign the relative configuration to the stereogenic centre formed during the Passerini reaction and to the one obtained through the cyclization, by comparing the two ^{1}H- NMR spectra. Since the OH group behaves as the nucleophile it is reasonable to assume that the configuration of the stereogenic centre (H-4 and H-4') is retained during the cyclization. Consequently, by comparison between the most significant coupling constants, it is possible to determine the two possible diastereoisomers (**3.23a** or **3.23b**).

1) Product **3.37a**: H-3 and H-4 couple, but their vicinal coupling constant is almost null (J= 0.66 Hz) because they describe a dihedral angle of about 90°. Therefore, in the ^{1}H-NMR spectrum, H-4 should be a singlet and H-3 a doublet because of coupling with H-2.
2) Product **3.38a**: H-3' and H- 4' couple, with a dihedral angle of around 0°, with a detectable coupling constant (J= 4.16 Hz). Thus, in the ^{1}H-NMR spectrum, H- 4' should be a doublet due to the coupling with H-3', and H-3' a doublet of doublets due to the coupling with H-4' and H- 2'.



Scheme 3.19 Stereochemical attribution of the stereogenic centres

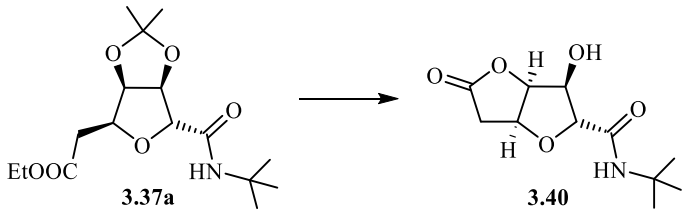
Part of the ^{1}H-NMR spectrum of the two products obtained is reported. It can be easily seen the two patterns described above. From these analyses, we could unambiguously demonstrate the relative configuration of the stereocentre in product **3.37a** and **3.38a** and, consequently, the configuration of **3.23a** and **3.23b**. Therefore, the configuration of the asymmetric carbon preferably produced in the Passerini reaction was elucidated (*R*).

From ^{1}H-NMR spectra was also possible to establish the configuration of the stereocentre generated during the cyclization. In both structures, **3.37a** and **3.38a**, H-1 and H-2 couple and their vicinal coupling constant is J = 4 Hz. Therefore, in the ^{1}H-NMR spectrum H-2 is a doublet of doublets due to the coupling with H-1 and H-3, and H-1 is a complex multiplet because of coupling with H-2 and the AB part of ABX system (ABX = CH_2CH). So, we can demonstrate the relative configuration (*S*) of this stereocentre.

3.2.4.3 Synthesis of bicyclic lactone

Another heterocycle that can be accessed from tetrahydrofurans, is lactone **3.40**. In fact, the tetrahydrofuran would afford the desired bicyclic ring system upon acidic removal of the acetonide protecting group followed by spontaneous lactonisation. This synthetic sequence was performed only on the (*S*, *R*) product (**3.37a**). However, it should be easily applicable to all the other stereoisomers. Two different procedures were carried out and they both gave good results (*Table 3.7*).

Table 3.7 Synthesis of bicyclic lactone



Entry	*Regents*	*Time*	*T*	*Solvent*	*Yield*
1[7a]	THF:TFA[a]:H_2O 2:1:1	8d	r.t.- reflux	THF (0.1M)	97%
2[15]	MeOH:TFA[a]:H_2O 1.6:1:1	3d	reflux	MeOH (0.44 M)	87%

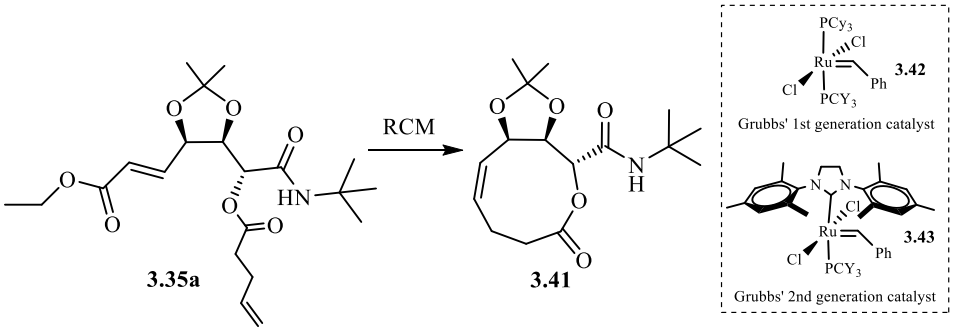
[a] TFA: trifluoroacetic acid

3.2.4.4 Ring closing metathesis (RCM)

One of the most useful methods for generating partially saturated heterocycles with different ring sizes is ring closing metathesis (RCM). In order to perform the

RCM, a precursor with two double bonds, one of them already present before the MCR and the other one introduced on either one of the reagents of the MCR (isocyanide or carboxylic acid), is required. Although the coupling of MCR with RCM has also been described by other groups, there were few examples in the literature of RCM that gives 9 membered unsaturated lactones[16] and none of these, starting from substituted alkenes. In fact, while RCM usually involves two terminal double bonds, in our case a terminal alkene and a substituted alkene are implicated (the α,β−unsaturated ester). However, some attempts to obtain the 9-membered rings were conducted, starting from the major diastereoisomer of Passerini product **3.35a**, employing Grubbs' 1st generation catalyst **3.42** and Grubbs' 2nd generation catalyst **3.43** in refluxing dry DCM (*Table 3.8*).

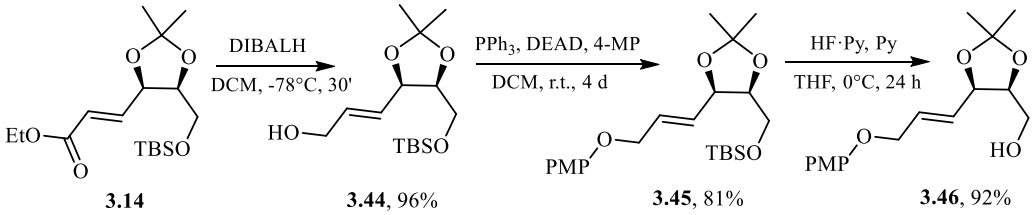
Table 3.8 Ring closing metathesis



Entry	*Catalyst*	*Solvent*	*T*	*Time*	*Yield*
1	**3.41**	DCM (0.004M)	reflux	3d	-
2	**3.42**	DCM (0.004M)	reflux	4h	-

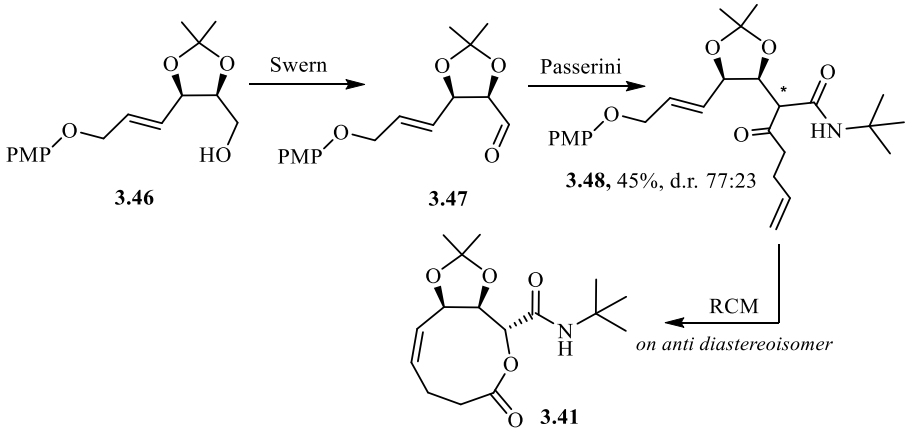
Unfortunately, in both reactions, the product was not obtained, and the starting material was recovered.

Due to the previous results, we decided to reduce the ester group and to attempt the RCM on the corresponding protected allylic alcohol (*Scheme 3.20*). The use of protecting groups is necessary to distinguish the two alcoholic functions.



Scheme 3.20 Synthesis of protected allyl alcohol

The protected allyl alcohol was prepared from α,β-unsaturated ester **3.14** in a 3-step sequence. At first, the ester was reduced with DIBAL-H[17] to give the desired substrate, allylic alcohol **3.44**. Then treatment of **3.43** under Mitsunobu conditions allowed to introduce the PMP protecting group and finally the TBS group was removed, using the previously optimized conditions. Compound **3.46** was then subjected to Swern oxidation, Passerini reaction and ring closing metathesis (*Scheme 3.21*).



Scheme 3.21 Ring closing metathesis

The reaction was performed with Grubbs' 1st generation catalyst and Grubbs' 2nd generation catalyst in refluxing dry DCM but the cyclized product was not obtained and the only compound isolated was the product derived from intermolecular metathesis (yield 15%). Probably, the higher steric requirements of the intramolecular reaction make the intermolecular reaction between two terminal olefins a competitive process even under high dilution (0.001 M).

3.3 Conclusion

In summary, thanks to their generality, versatility and ease of combination with various transformations, MCRs can be considered a great approach in diversity-oriented synthesis and exploration of chemical space.

In the present work, we report the coupling of biocatalysis, Passerini three component reaction and cyclizations to access a variety of functionalized oxygen heterocycles with control over the stereochemical outcome. In particular, we worked on a promising achiral building block, derived from renewable sources, with the aim of enhancing its value by increasing complexity and appendage and scaffold diversity.

3.4 Experimental Section

3.4.1 General remarks

NMR spectra were taken at room temperature in $CDCl_3$ or in d_6-DMSO at 300 MHz (^1H), and 75 MHz (^{13}C), using, as internal standard, TMS (^1H-NMR: 0.000 ppm) or the central peak of $CDCl_3$ (^{13}C: 77.160 ppm). Chemical shifts are reported in ppm (δ scale). Coupling constants are reported in Hertz. Resonances are described as s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet) and m (multiplet) or combinations thereof. Peak assignments were made with the aid of gCOSY and gHSQC experiments. In ABX system, the proton A is considered upfield and B downfield.

GC-MS were carried out using an HP-1 column (12 m long, 0.2 mm wide), electron impact at 70 eV, and a mass temperature of about 170 °C. Only m/z > 33 were detected. All analyses were performed (unless otherwise stated) with a constant He flow of 1.0 ml/min with initial temp. of 70 °C, init. time 2 min, rate 20 °C/min, final temp. 260 °C, inj. temp. 250 °C, det. temp. 280 °C.

HPLC analyses for the determination of enantiomeric ratios were performed on a Daicel Chiral Pak AD 250x3.6 mm column, at 25–28 °C with a flow of 1 mL/min (UV detection at λ = 220 nm).

HPLC-MS analyses were performed on Synergi Hydro RP 150x3 mm column, at 30 °C with a flow of 0.5 mL/min (where not otherwise stated). HRMS: samples, provided at 10mM in DMSO, were analysed on a UPLC Acquity system coupled to a Synapt G2 QToF mass spectrometer. MS signals were acquired from 50 to 1200 m/z both ESI positive and ESI negative ionization mode.

IR spectra were recorded directly on solid, oil, or foamy samples with the ATR (attenuated total reflectance) technique, using a FT Perkin Elmer Spectrum 65 spectrophotometer.

Optical rotations were measured with a JASCO P-2000 digital polarimeter, using the sodium D line as the light source. The specific rotation $[\alpha]^D$ was calculated as: $[\alpha]_D = \alpha * 100 / c * l$ (α = observed rotation of solution; l = length of polarimeter tube in decimetres, c = concentration of the solution in g/100 mL in $CHCl_3$).

Melting points were determined with an electrothermal apparatus (Büchi B-535).

TLC analyses were carried out on silica gel plates and viewed at UV (254 nm) and developed with Hanessian stain (dipping into a solution of $(NH_4)_4MoO_4 \cdot 4H_2O$ (21 g) and $Ce(SO_4)_2 \cdot 4H_2O$ (1 g) in H_2SO_4 (31 mL) and H_2O (469 mL) and warming).

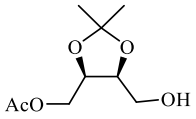
Rf values were measured after an elution of 7-9 cm.

Column chromatographies were done with the "flash" methodology using 220-400 mesh silica.

All reactions using dry solvents were carried out under a nitrogen or argon atmosphere.

3.4.2 Experimental procedures

3.4.2.1 Synthesis of chiral building blocks

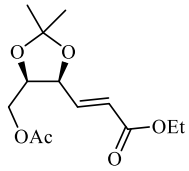


((4R,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl) methyl acetate 3.2

Prepared as previously reported by us[7a].

Colourless liquid, yield 96%, e.e. 96%, conversion: 51%.

$[\alpha]^{24}_{D}$= + 16.69 (c = 1.095, $CHCl_3$)



Ethyl (E)-3-((4S,5R)-5-(acetoxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)acrylate 3.9

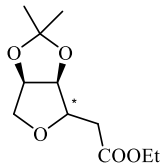
Swern oxidation

To a solution of DMSO (87 μL, 1.22 mmol) in dry CH_2Cl_2 (2.9 ml), at –78 °C under nitrogen atmosphere, a solution of oxalyl chloride in dry CH_2Cl_2 (1.43 M, 515 μL) was added. Upon completion of the addition, the mixture was stirred at -78 °C for 10 min. A solution of alcohol **3.3** (100 mg, 0.49 mmol) in dry CH_2Cl_2 (2 ml) was added dropwise, and the solution was stirred for 10 min at –78 °C. Then Et_3N (320 μL, 2.30 mmol) was added and the solution was stirred for 1 h. The reaction was quenched by addition of 5% aq. $(NH_4)H_2PO_4$ (10 mL + 1 N HCl solution) to have a final pH = 4 and extracted with Et_2O (3 x 10 mL). The combined organic layers were washed with $NaHCO_3$ 5% solution (10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated to afford aldehyde **3.7** as a yellow oil, which was immediately used for the next reaction without further purification.

Horner – Wadsworth - Emmons reaction

To a suspension of LiCl (31 mg, 0.74 mmol) and 3Å molecular sieves (10 mg/0.1 mmol) in dry MeCN (5.4 mL) triethyl phosphonoacetate (165 μL, 0.73 mmol) diisopropylethylamine (84 μL, 0.49 mmol) and the crude aldehyde were added. The reaction was stirred at room temperature for 28 h. The reaction was filtered on celite and then extracted with NH_4Cl solution (10 mL) and Et_2O (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (ETP: Et_2O 7:3) to afford the α,β-unsaturated ester (108 mg, 81% yield in 2 steps) as a mixture of diastereoisomers (d.r. = 95:5 *E: Z*, calculated by ^{1}H-NMR analysis on the crude product).

3.9 E diastereoisomer: pale yiellow oil; Rf = 0.25 (ETP: Et_2O 7:3), developed with Hanessian stain.

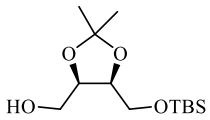


Ethyl 2-((3aS,6aR)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)acetate 3.11

Compound **3.9** (50 mg, 0.18 mmol) was solubilized in EtOH (1 mL). H_2O (200 μL) and Et_3N (200 μL) were added and the reaction was stirred for 6 days at room temperature. The reaction was quenched with 10 mL of NH_4Cl (sat) and extracted with Et_2O (3 x 10 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography on silica gel (from ETP: Et_2O 6:4 to Et_2O + 1% EtOH) to give 26 mg (61% yield) of **3.11** as a colourless oil. Analysis of the crude product by GC-MS revealed a diastereomeric ratio of 74:26.
Rf = 0.30 (ETP: Et_2O 1:1), developed with Hanessian stain.

Legend for NMR spectra: M= major diastereoisomer, m= minor diastereoisomer.
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS) mixture of the two diasteroisomers (M + m) not separable: δ = 4.85- 4.82 (m, 1H, C<u>H</u>CH_2O, m), 4.79 (dd, J = 6.2, 3.5 Hz, 1H, C<u>H</u>CH_2O, M), 4.71 (dd, J = 6.1, 3.6 Hz, 1H, C<u>H</u>CHO, M), 4.57 (dd, 6.3, 1.6 Hz, 1H, C<u>H</u>CHO, m), 4.46 (td, J = 7.2, 1.7 Hz, 1H, CHC<u>H</u>O, m) 4.24 – 4.11 (m, 4H, C<u>H_2</u>CH_3, M and m), 4.01 (d, J = 10.9 Hz, 1H, CHC<u>H_2</u>O, M), 3.96 (d, J = 1.2 Hz, 1H, CHC<u>H_2</u>O, m), 3.87 (dd, J = 6.5, 3.9 Hz, 1H, CHC<u>H</u>O, M), 3.85 – 3.83 (m, 1H. CHC<u>H_2</u>O, m), 3.49 (dd, J = 10.8, 3.6 Hz, 1H, CHC<u>H_2</u>O, M), 2.81 and 2.78 (part AB of ABX syst., J_{AB} = 14.47, J_{AX} = 2.12, J_{BX} = 2.41. Hz, 2 H, CHC<u>H_2</u>C=O, M), 2.51 and 2.46 (part AB of ABX syst., J_{AB} = 12.13, J_{AX} = 1.07, J_{BX} = 0.37 Hz, 2 H, CHC<u>H_2</u>C=O, m), 1.52 (s, 3H, CH_3 of *i*Pr, m), 1.48 (s, 3H, CH_3 of *i*Pr, M), 1.33 (s, 6H, CH_3 of *i*Pr (M and m)) 1.28 (t, J= 7.1 Hz, 3H, CH_2C<u>H_3</u>, M) 1.27 (t, J = 7.1, Hz, 3H, CH_2C<u>H_3</u>, m). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS) mixture of the two diasteroisomers (M + m) not separable: δ = 171.32 (C=O, M), 170.52 (C=O, m), 113.14 (<u>C</u>$(CH_3)_2$, m), 112.33 (<u>C</u>$(CH_3)_2$, M), 84.64 (<u>C</u>HCHO m), 81.25 (<u>C</u>HCH_2O, M), 81.21 (<u>C</u>HCH_2O, m), 81.09 (CH<u>C</u>HO, m), 80.93 (<u>C</u>HCHO, M), 78.48 (CH<u>C</u>HO, M), 72.80 (CH<u>C</u>H_2O, M), 72.42 (CH<u>C</u>H_2O, m), 60.96 (<u>C</u>H_2CH_3, m), 60.79 (<u>C</u>H_2CH_3, M), 36.56 (CH<u>C</u>H_2C=O, m), 33.94 (CH<u>C</u>H_2C=O, M), 26.75 (CH_3 of *i*Pr, m), 26.16 (CH_3 of *i*Pr, M), 25.17 (CH_3 of *i*Pr, m), 25.03 (CH_3 of *i*Pr, M), 15.42 (CH_2<u>C</u>H_3, M), 14.31 (CH_2<u>C</u>H_3, m). *IR* mixture of the two diasteroisomers (M + m) not separable ν (cm-1) = 2983, 2937, 2863, 1732, 1458, 1372, 1334, 1305, 1269, 1207, 1179, 1163, 1133, 1090, 1056, 1027, 1004, 993, 975, 952, 916, 892, 860, 815, 722, 695. *GC-MS* (initial temp. 70 °C): Rt_1 = 6.29 min: m/z 216 (M+ -15, 10.8%), 216 (11), 215 (100), 186 (5,1), 185 (59), 172 (15), 155 (48), 127 (48), 126 (6,6), 117 (5,7), 115 (5,6), 113 (15), 109 (11), 101 (15), 99 (18), 98 (5,3), 97 (5,9), 89 (12), 88 (9,1), 86 (5,7), 85 (57), 84 (8,9), 83 (15), 82 (6,8), 81 (83), 71 (37), 70 (7,0), 69 (10), 60 (7,9), 59 (42), 58 (7,9), 57 (11), 56 (7,0), 55 (17), 45 (5,3), 43 (50), 42 (5,3), 41 (8,5); Rt_2 = 6.46 min: m/z 216 (M+ -15, 7.5%), 216 (7,5), 215 (84), 200 (17), 185 (13), 172 (7,6), 155 (17), 144 (5,8), 143 (69), 127 (54), 126 (7,8), 113 (8,7), 109 (16), 101 (11), 99 (12), 98 (6,8), 97 (7,8), 89 (11), 88 (9,2), 86 (7,1), 85

(70), 84 (8,0), 83 (7,7), 82 (8,1), 81 (100), 71 (31), 70 (8,6), 69 (7,9), 61 (5,0), 60 (8,2), 59 (40), 58 (7,3), 57 (21), 56 (5,6), 55 (12), 43 (53), 42 (5,5), 41 (8,5).



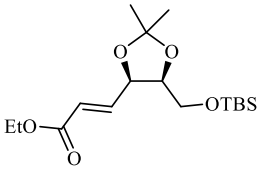
((4R,5S)-5-(((tert-butyldiphenylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol 3.12

Prepared as previously reported in literature[10].

Pale yellow oil, yield 91%.

$[\alpha]^{23}_D$= + 3.73 (c = 1.03, $CHCl_3$).

HRMS (ESI+): m/z calcd for $C_{13}H_{28}O_4Si$ [M+Na]+: 299.1655; found 299.1661.



Ethyl 3-((4R,5S)-5-(((tertbutyldimethylsilyl)oxy) methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)acrylate 3.14

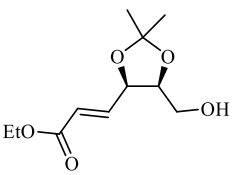
Swern oxidation

Aldehyde **3.13** was prepared employing the same procedure of **3.7** from alcohol **3.12** (916 mg, 3.31 mmol).

Horner – Wadsworth - Emmons reaction

To a suspension of sodium hydride (60% in oil, 186 mg, 3.64 mmol) in dry THF (11.5 mL) was added triethyl phosphonoacetate (986 μL, 3.97 mmol) at 0 °C under argon atmosphere. The mixture was stirred at the same temperature for 30 min. To the mixture, a solution of aldehyde **3.13** (3.31 mmol) in dry THF (8 mL) was added at -78 °C. The mixture was stirred at the same temperature for 10 min, warmed to room temperature and stirred for 45 min. The reaction was diluted with AcOEt (30 mL) and poured into saturated aqueous NH_4Cl solution (30 mL). The aqueous phase was extracted with AcOEt (2 x 20). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography (ETP: Et_2O 95:5) to afford the α, β-unsaturated ester (1.071 g, 94% yield in 2 steps) as a mixture of diastereoisomers (d.r. = 97:3 *E: Z*, calculated by ^{1}H-NMR analysis on the crude product). The analytical data conform to those reported in literature[10].

3.14 *E* diastereoisomer: colourless oil; Rf = 0.88 (ETP: Et_2O 1:1), developed with Hanessian stain. $[\alpha]^{24}_D$= + 12.03 (c = 0.97, $CHCl_3$).

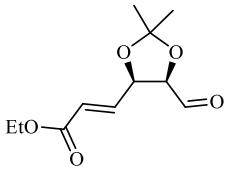


Ethyl (E)-3-((4R,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl) acrylate 3.3

Compound **3.14** (803 mg, 2.33 mmol) was dissolved in 16 mL of dry THF and 8.6 mL of pyridine (106.10 mmol) under nitrogen atmosphere. Then HF·pyridinium complex (70%, 2.1 mL, 81.59 mmol) was added to the solution at 0°C and the reaction was stirred at the same temperature for 3 days. The reaction was quenched with 20 mL of NH_4Cl (sat) and extracted with Et_2O (3 x 20 mL). The combined organic layers were washed with water (4 x 25 mL) and brine (20 mL), dried (Na_2SO_4) and concentrated. The residue was

purified by flash column chromatography on silica gel (ETP: Et_2O 3:7) to give 532 mg (99% yield) of **3.3** as a colourless oil. The analytical data conform to those reported in literature[18].
Rf = 0.25 (PE/Et_2O 3:7), developed with Hanessian stain.
$[\alpha]^{24}_D$= - 33.32 (c = 1.14, $CHCl_3$).

3.4.2.2 Synthesis of Passerini products

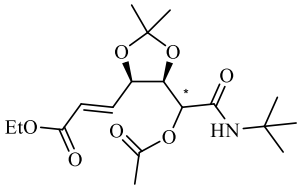


Ethyl (E)-3-((4R,5R)-5-formyl-2,2-dimethyl-1,3-dioxolan-4-yl)acrylate 3.22

To a solution of DMSO (105 μL, 1.47 mmol) in dry CH_2Cl_2 (3.4 ml), at –78 °C under nitrogen atmosphere, a solution of oxalyl chloride in dry CH_2Cl_2 (1.43 M, 949 μL) was added. Upon completion of the addition, the mixture was stirred at -78 °C for 10 min. A solution of alcohol **3.3** (125 mg, 0.54 mmol) in dry CH_2Cl_2 (2 ml) was added dropwise, and the solution was stirred for 10 min at –78 °C. Then Et_3N (350 μL, 2.52 mmol) was added and the solution was stirred for 1 h. The reaction was quenched by addition of 5% aq. $(NH_4)H_2PO_4$ (10 mL + 1 N HCl solution) to have a final pH = 4 and extracted with Et_2O (3 x 10 mL). The combined organic layers were washed with $NaHCO_3$ 5% solution (10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated to afford 129 mg of **3.22** as a yellow oil. The crude product was directly employed in the next Passerini reaction.

General procedure A for Passerini reaction under classical conditions
A solution of crude aldehyde in dry THF (1 M) was placed at 20°C under nitrogen atmosphere and was treated with the carboxylic acid (1.1 eq) and the isocyanide (1.1 eq). The crude product was concentrated and purified by flash column chromatography on silica gel.

General procedure B for Passerini reaction with $ZnBr_2$
A solution of $ZnBr_2$ (0.4 eq) in dry THF (1 M) was placed at 20°C under nitrogen atmosphere. The crude aldehyde (1 eq), the carboxylic acid (1.1 eq) and the isocyanide (1.1 eq) were added. The resulting solution was stirred at 20°C for 16-25 h. The reaction was monitored by GC-MS, quenched with NH_4Cl (sat) and extracted with ethyl acetate. The combined organic layers were washed with $NaHCO_3$ 5% solution, dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography on silica gel.



Ethyl (E)-3-((4R,5R)-5-(1-acetoxy-2-(tert-butylamino)-2-oxoethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)acrylate 3.23

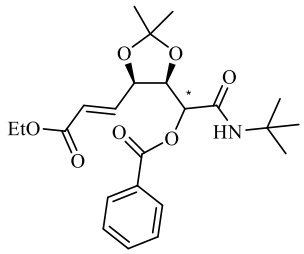
Firstly, prepared from crude aldehyde **3.22** (0.25 mmol), acetic acid (16 μL, 0.27 mmol) and *tert*-butylisocyanide (31

μL, 0.27 mmol) according to procedure A. The crude was purified by column chromatography (ETP:DCM:Et_2O 3:1:1) affording **3.23_*anti*** and **3.23_*syn*** (d.r. = 51 : 49 calculated by ^{1}H NMR analysis on the crude product) (90 mg, 97%) as a mixture of diastereoisomers.
Then prepared from crude aldehyde **3.22** (0.52 mmol), acetic acid (33 μL, 0.57 mmol), *tert*-butylisocyanide (65 μL, 0.57 mmol) and $ZnBr_2$ (47 mg, 0.21 mmol) according to procedure B. The crude product was purified by column chromatography (ETP:DCM:Et_2O 3:1:1) affording **3.23_*anti*** and **3.23_*syn*** (d.r. = 73: 27 calculated by ^{1}H NMR analysis on the crude product) (151 mg, 78%) as a mixture of diastereoisomers.

3.23_*anti* colourless oil; R_f = (0.68, ETP: Et_2O 2:8) developed with Hanessian stain.
$[\alpha]^{24}_D$= - 50.26 (c = 0.815, $CHCl_3$).
^{1}H NMR (300 MHz, CDCl3, 25 °C,TMS): δ = 6.88 (dd, J = 15.6, 5.4 Hz, 1H, CHC<u>H</u>=CH), 6.10 (dd, J = 15.6, 1.6 Hz, 1H, O=CC<u>H</u>=CH), 5.81 (broad s, 1H, NH), 4.87 (ddd, J = 6.7, 5.4, 1.6 Hz, 1H, CHC<u>H</u>O), 4.84 (d, J = 7.9 Hz, 1H, C<u>H</u>OAc), 4.65 (dd, J = 7.9, 6.5 Hz, 1H, C<u>H</u>CHOAc), 4.19 (q, J = 7.1 Hz, 2H, C<u>H$_2$</u>CH$_3$), 2.10 (s, 3H, C<u>H$_3$</u>of OAc), 1.51 (s, 3H, *i*Pr), 1.38 (s, 3H, *i*Pr), 1.32 (s, 9H, 3 CH$_3$ of *t*Bu), 1.27 (t, J = 7.1 Hz, 3H, CH$_2$C<u>H$_3$</u>). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 169.54 (C=ONH), 166.11 (<u>C</u>=O), 165.95 (<u>C</u>=O), 141.46 (CH<u>C</u>H=CH), 122.59 (O=C<u>C</u>H=CH), 109.78 (<u>C</u>(CH$_3$)$_2$), 76.55 (<u>C</u>HCHOAc), 76.24 (CH<u>C</u>HO), 71.41 (<u>C</u>HOAc), 60.72 (<u>C</u>H$_2$CH$_3$), 51.82 (<u>C</u>(CH$_3$)$_3$), 28.69 (3 CH$_3$ of *t*Bu), 27.70 (CH$_3$ of *i*Pr), 25.14 (CH$_3$ of *i*Pr), 20.73 (CH$_3$ of OAc), 14.37 (CH$_2$<u>C</u>H$_3$). *IR* ν (cm^{-1}) = 3355, 2981, 2938, 1751, 1719, 1682, 1525, 1456, 1368, 1303, 1253, 1210, 1179, 1161, 1122, 1060, 1033, 982, 938, 883, 861, 795. *GC-MS* (initial temp. 70 °C): R_t 9.89 min: m/z 357 (M+-15, 0.2%), 198 (5.1), 172 (6.8), 171 (8.6), 170 (12), 154 (7.3), 152 (8.6), 151 (7.0), 143 (11), 131 (5.3), 130 (17), 129 (6.7), 126 (14), 125 (13), 113 (6.1), 112 (30), 109 (7.9), 108 (5.5), 101 (8.1), 97 (24), 85 (15), 84 (43), 83 (5.6), 81 (10), 69 (6.0), 59 (17), 58 (43), 57 (52), 56 (5.1), 55 (10), 43 (100), 42 (6.3), 41 (23), 39 (9.9). *HRMS* (ESI+): m/z calcd for $C_{18}H_{29}NO_7$ $[M+Na]^+$: 393.1842; found: 393.1835.

3.23_*syn* colourless oil; R_f = (0.79, ETP: Et_2O 2:8) developed with Hanessian stain.
$[\alpha]^{24}_D$= - 62.52 (c = 0.90, $CHCl_3$).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 6.74 (dd, J = 15.6, 5.6 Hz, 1H, CHC<u>H</u>=CH), 6.03 (dd, J = 15.6, 1.6 Hz, 1H, O=CC<u>H</u>=CH), 5.79 (broad s, 1H, NH), 4.87 (d, J = 5.7 Hz, 1H, C<u>H</u>OAc), 4.82 (ddd, J = 7.1, 5.6, 1.6 Hz, 1H, CHC<u>H</u>O), 4.68 (dd, J = 7.0, 5.7 Hz, 1H, C<u>H</u>CHOAc), 4.13 (q, J = 7.2 Hz, 1H, C<u>H$_2$</u>CH$_3$), 2.08 (s, 3H, C<u>H$_3$</u> of OAc), 1.46 (s, 3H, *i*Pr), 1.32 (s, 3H, *i*Pr), 1.25 (s, 9H, 3 CH$_3$ of *t*Bu), 1.22 (t, J = 7.1 Hz, 1H, CH$_2$C<u>H$_3$</u>). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 170.29 (C=ONH), 166.31 (<u>C</u>=O), 165.75 (<u>C</u>=O), 141.97 (CH<u>C</u>H=CH), 123.59 (O=C<u>C</u>H=CH), 109.82 (<u>C</u>(CH$_3$)$_2$), 76.84 (<u>C</u>HCHOAc), 75.99 (CH<u>C</u>HO), 72.94 (<u>C</u>HOAc), 60.74 (<u>C</u>H$_2$CH$_3$), 51.71 (<u>C</u>(CH$_3$)$_3$), 28.60 (3 CH$_3$ of *t*Bu), 27.29 (CH$_3$ of

*i*Pr), 25.29 (CH_3 of *i*Pr), 21.09 (CH_3 of OAc), 14.35 ($CH_2\underline{C}H_3$). *IR* ν (cm^{-1}) = 3361, 2981, 2937, 2875, 1751, 1719, 1682, 1525, 1456, 1368, 1303, 1254, 1210, 1178, 1161, 1121, 1060, 1033, 983, 938, 883, 862, 795, 661. *GC-MS* (initial temp. 70 °C): R_t 9.79 min: m/z 357 (M+-15, 0.8%), 198 (12), 172 (6.0), 171 (8.3), 170 (9.1), 154 (5.1), 152 (9.9), 151 (5.2), 143 (8.6), 198 (12), 172 (6.0), 171 (8.3), 170 (9.1), 154 (5.1), 152 (9.9), 151 (5.2), 143 (8.6), 131 (6.8), 130 (19), 129 (7.2), 126 (12), 125 (11), 113 (5.7), 112 (27), 109 (6.0), 108 (5.3), 101 (8.5), 97 (22), 85 (15), 84 (36), 83 (5.4), 81 (9.0), 69 (6.3), 59 (16), 58 (44), 57 (49), 55 (11), 43 (100), 42 (6.3), 41 (22), 39 (9.1). *HRMS* (ESI+): m/z calcd for $C_{18}H_{29}NO_7$ $[M+Na]^+$: 393.1842; found: 393.1830.



2-(tert-butylamino)-1-((4R,5R)-5-((E)-3-ethoxy-3-oxoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxoethyl benzoate 3.29

Firstly, prepared from crude aldehyde **3.22** (0.74 mmol), benzoic acid (100 mg, 0.82 mmol) and *tert*-butylisocyanide (92 μL, 0.82 mmol), according to procedure A. The crude product was purified by column chromatography (ETP:DCM:Et_2O 3:1:1) affording **3.29*_anti*** and **3.29*_syn*** (d.r. = 46 : 54 calculated by HPLC analysis and confirmed by ^{1}H-NMR analysis on the crude product) (314 mg, 97%) as a mixture of diastereoisomers.

Then prepared from crude aldehyde (0.65 mmol), benzoic acid (87 mg, 0.71 mmol), *tert*-butylisocyanide (81 μL, 0.71 mmol) and $ZnBr_2$ (59 mg, 0.26 mmol) according to procedure B. The crude product was purified by column chromatography (ETP:DCM:Et_2O 3:1:1) affording **3.29*_anti*** and **3.29*_syn*** (d.r. = 76: 24 calculated by HPLC analysis and confirmed by ^{1}H-NMR (73:27) analysis on the crude product) (167 mg, 59%) as a mixture of diastereoisomers.

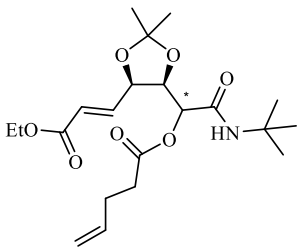
3.29*_anti* white solid; R_f = (0.64, ETP: Et_2O 4:6) developed with Hanessian stain. $[\alpha]^{24}_D$= - 53.53 (c = 0.97, $CHCl_3$). m.p. 103.5-107.1 °C (CH_2Cl_2).

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 8.11 – 8.01 (m, 2H, 2 CH of Ph), 7.68 – 7.55 (m, 1H, CH of Ph), 7.54 – 7.42 (m, 2H, 2 CH of Ph), 7.07 – 6.94 (m, 1H, CHC$\underline{H}$=CH), 6.15 (dd, J = 15.6, 1.0 Hz, 1H, O=CC$\underline{H}$=CH), 5.90 (broad s, 1H, NH), 5.56 – 5.47 (m, 1H, C$\underline{H}$Bz), 5.00 – 4.91 (m, 2H, CHC$\underline{H}$O and C$\underline{H}$CHBz), 4.10 (qd, J = 7.1, 1.8 Hz, 2H, $\underline{C}H_2CH_3$), 1.39 (s, 6H 2 CH_3 of *i*Pr), 1.34 (s, 9H, 3 CH_3 of *t*Bu), 1.19 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 166.09 (C=ONH), 165.78 (C=O), 165.19 (C=O), 142.37 (CH$\underline{C}$H=CH), 133.84 ($\underline{C}$H of Ph), 130.07 (2 $\underline{C}$H of Ph), 128.92 (C quat. of Ph), 128.75 (2 $\underline{C}$H of Ph), 123.17 (O=C$\underline{C}$H=CH), 109.64 ($\underline{C}(CH_3)_2$), 76.13 (CH$\underline{C}$HO and $\underline{C}$HBz) 71.98 ($\underline{C}$HCHBz), 60.59 ($\underline{C}H_2CH_3$), 51.86 ($\underline{C}(CH_3)_3$), 28.60 (3 CH_3 of *t*Bu), 27.33 (CH_3 of *i*Pr), 24.94 (CH_3 of *i*Pr), 14.28 ($CH_2\underline{C}H_3$). *IR* ν (cm^{-1}) = 3338, 3077, 2977, 1712, 1669, 1602, 1535, 1453, 1368, 1294, 1253, 1238, 1218, 1179, 1161, 1114, 1068, 1047, 1029, 1001, 989, 881, 819, 804, 787, 760, 709, 686, 671, 626. *GC-MS* (initial temp. 70 °C): R_t

11.91 min: m/z 418 (M+-15, 2.2%), 130 (5.2), 112 (8.9), 106 (7.0), 105 (100), 84 (12), 77 (16), 58 (6.7), 57 (16), 43 (7.8), 41 (7.1). *HRMS* (ESI+): m/z calcd for $C_{23}H_{31}NO_7$ $[M+Na]^+$: 456.1999; found: 456.1995.

3.29_*syn* white solid; R_f = (0.59, ETP: Et_2O 4:6) developed with Hanessian stain. $[\alpha]^{24}_D$= - 69.80 (c =1.00, $CHCl_3$). m.p. 99.6 - 103.8 °C (CH_2Cl_2).

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 8.16 – 8.03 (m, 2H, 2 CH of Ph), 7.66 – 7.56 (m, 1H, CH of Ph), 7.54 – 7.41 (m, 2H, 2 CH of Ph), 6.83 (dd, J = 15.7, 4.9 Hz, 1H, CHC<u>H</u>=CH), 6.06 (dd, J = 15.7, 1.4 Hz, 1H, O=CC<u>H</u>=CH), 5.96 (broad s, 1H, NH), 5.22 (d, J = 4.7 Hz, 1H, C<u>H</u>Bz), 5.00 – 4.88 (m, 2H, CHC<u>H</u>O and C<u>H</u>CHBz), 4.03 (q, J = 7.1 Hz, 2H, <u>C</u>H_2CH_3), 1.59 (s, 3H, CH_3 of *i*Pr), 1.42 (s, 3H, CH_3 of *i*Pr), 1.31 (s, 9H, 3 CH_3 of *t*Bu), 1.14 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 166.46 (C=ONH), 165.75 (C=O), 165.47 (C=O), 141.75 (CH<u>C</u>H=CH), 133.82 (<u>C</u>H of Ph), 130.09 (2 <u>C</u>H of Ph), 129.12 (C quat. of Ph), 128.73 (2 <u>C</u>H of Ph), 123.58 (O=C<u>C</u>H=CH), 109.72 (<u>C</u>$(CH_3)_2$), 77.09 (CH<u>C</u>HO), 75.93 (<u>C</u>HCHBz), 73.42 (<u>C</u>HBz), 60.50 (<u>C</u>H_2CH_3), 51.71 (<u>C</u>$(CH_3)_3$), 28.62 (3 CH_3 of *t*Bu), 27.30 (CH_3 of *i*Pr), 25.10 (CH_3 of *i*Pr), 14.16, ($CH_2\underline{C}H_3$). *IR* ν (cm^{-1}) = 3362, 2981, 2965, 2937, 1730, 1713, 1688, 1603, 1586, 1543, 1495, 1454, 1381, 1364, 1317, 1296, 1256, 1213, 1181, 1161, 1134, 1099, 1060, 1039, 987, 947, 914, 885, 851, 798, 765, 716, 688, 676, 633, 610. *GC-MS* (initial temp. 70 °C): R_t 12.23 min: m/z 418 (M+-15, 0.9%), 171 (9.4), 130 (5.9), 112 (8.8), 106 (8.8), 105 (100), 84 (12), 77 (15), 58 (6.6), 57 (14), 43 (6.2), 41 (5.3). *HRMS* (ESI+): m/z calcd for $C_{23}H_{31}NO_7$ $[M+Na]^+$: 456.1999; found: 456.1995.



2-(tert-butylamino)-1-((4R,5R)-5-((E)-3-ethoxy-3-oxoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxoethyl pent-4-enoate 3.35

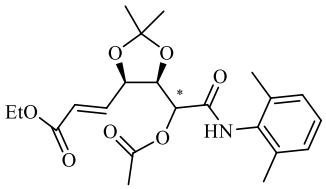
Firstly, prepared from crude aldehyde **3.22** (0.434 mmol), pentenoic acid (48 μL, 0.48 mmol) and *tert*-butylisocyanide (54 μL, 0.48 mmol) according to procedure A. The crude product was purified by column chromatography ((ETP:DCM:Et_2O 3:1:1) affording **3.35_*anti*** and **3.35_syn** (d.r. = 47 : 53 calculated by HPLC analysis on the crude product) (117 mg, 66%) as a mixture of diastereoisomers.

Then prepared from crude aldehyde 3.22 (0.650 mmol), pentenoic acid (72 μL, 0.71 mmol), *tert*-butylisocyanide (81 μL, 0.71 mmol) and $ZnBr_2$ (59 mg, 0.26 mmol) according to procedure B. The crude product was purified by column chromatography ((ETP:DCM:Et_2O 3:1:1) affording **3.35_*anti*** and **3.35_*syn*** (d.r. = 77 : 23 calculated by HPLC analysis on the crude product) (142 mg, 53%) as a mixture of diastereoisomers.

3.35_*anti* colourless oil; R_f = (0.70, ETP: Et_2O 4: 6) developed with Hanessian stain. $[\alpha]^{24}_D$= - 22.22 (c = 0.99, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 6.89 (dd, J = 15.7, 5.7 Hz, 1H, CHC<u>H</u>=CH), 6.10 (dd, J = 15.6, 1.6 Hz, 1H, O=CC<u>H</u>=CH), 5.91 – 5.73 (m, 2H, NH and CH_2=C<u>H</u>CH_2), 5.12 – 4.98 (m, 3H, C<u>H_2</u>=CHCH_2 and C<u>H</u>OC=O), 4.87 (ddd, J = 6.9, 5.7, 1.6 Hz, 1H, CHC<u>H</u>O), 4.72 (t, J = 6.8 Hz, 1H, C<u>H</u>CHOC=O), 4.19 (q, J = 7.1 Hz, 2H, C<u>H_2</u>CH_3), 2.54 – 2.46 (m, 2H, CH_2C<u>H_2</u>C=O), 2.43 – 2.33 (m, 2H, C<u>H_2</u>CH_2C=O), 1.51 (s, 3H, CH_3 of *i*Pr), 1.38 (s, 3H, CH_3 of *i*Pr), 1.32 (s, 9H, 3 CH_3 of *t*Bu), 1.28 (t, J = 7.1 Hz, 3H, CH_2C<u>H_3</u>). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 171.53 (C=ONH), 166.06 (C=O), 165.92 (C=O), 141.99 (CH<u>C</u>H=CH), 136.42 (CH_2=<u>C</u>HCH_2), 122.76 (O=C<u>C</u>H=CH), 115.97 (<u>C</u>H_2=CHCH_2), 109.64 (<u>C</u>$(CH_3)_2$), 76.68 (<u>C</u>HCHOC=O), 76.17 (CH<u>C</u>HO), 71.40 (CHOC=O), 60.68 (<u>C</u>H_2CH_3), 51.80 (<u>C</u>$(CH_3)_3$), 33.18 (CH_2<u>C</u>H_2C=O), 28.64 (3 CH_3 of *t*Bu), 28.49 (<u>C</u>H_2CH_2C=O), 27.60 (CH_3 of *i*Pr), 25.03 (CH_3 of *i*Pr), 14.38 (CH_2<u>C</u>H_3). *IR* ν (cm^{-1}) = 3385, 2987, 2966, 2940, 1732, 1714, 1687, 1534, 1454, 1380, 1367, 1300, 1255, 1222, 1160, 1128, 1108, 1070, 1043, 1003, 984, 957, 931, 914, 881, 810, 790, 762, 744, 691, 666, 631, 603. *GC-MS* (initial temp. 70 °C): R_t 10.75 min: m/z 396 (M+-15, 0.1%), 170 (7.4), 154 (6.1), 152 (5.4), 151 (5.9), 130 (11), 129 (5.8), 126 (7.4), 125 (10), 112 (25), 97 (15), 85 (7.9), 84 (40), 83 (58), 81 (8.8), 69 (5.3), 59 (13), 58 (33), 57 (51), 56 (8.9), 55 (100), 54 (6.6), 53 (7.1), 43 (25), 42 (6.2), 41 (27), 39 (15). *HRMS* (ESI+): m/z calcd for $C_{21}H_{33}NO_7$ $[M+Na]^+$: 433.2155; found: 433.2153.

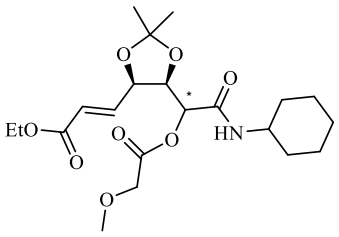
3.35_*syn* colourless oil; R_f = (0.67, ETP: Et_2O 4: 6) developed with Hanessian stain. $[\alpha]^{20}_D$= - 62.20 (c = 0.54, $CHCl_3$).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 6.81 (dd, J = 15.6, 5.6 Hz, 1H, CHC<u>H</u>=CH), 6.09 (dd, J = 15.6, 1.6 Hz, 1H, O=CC<u>H</u>=CH), 5.91 – 5.75 (m, 2H, NH and CH_2=C<u>H</u>CH_2), 5.15 – 5.00 (m, 2H, C<u>H_2</u>=CHCH_2), 4.95 (d, J = 5.8 Hz, 1H, C<u>H</u>OC=O), 4.89 (ddd, J = 7.1, 5.6, 1.6 Hz, 1H, CHC<u>H</u>O), 4.75 (dd, J = 7.0, 5.8 Hz, 1H, C<u>H</u>CHOC=O), 4.19 (q, J = 7.1 Hz, 2H, C<u>H_2</u>CH_3), 2.55 – 2.48 (m, 2H, CH_2C<u>H_2</u>C=O), 2.45 – 2.35 (m, 2H, C<u>H_2</u>CH_2C=O), 1.52 (s, 3H, CH_3 of *i*Pr), 1.38 (s, 3H, CH_3 of *i*Pr), 1.30 (s, 9H, 3 CH_3 of *t*Bu), 1.27 (t, J = 7.1 Hz, 3H, CH_2C<u>H_3</u>). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 172.32 (C=ONH), 166.36 (C=O), 165.72 (C=O), 142.00 (CH<u>C</u>H=CH), 136.33 (CH_2=<u>C</u>HCH_2), 123.60 (O=C<u>C</u>H=CH), 116.12 (<u>C</u>H_2=CHCH_2), 109.78 (<u>C</u>$(CH_3)_2$), 76.88 (<u>C</u>HCHOC=O), 76.04 (CH<u>C</u>HO), 72.92 (C<u>H</u>OC=O), 60.72 (<u>C</u>H_2CH_3), 51.70 (<u>C</u>$(CH_3)_3$), 33.50 (CH_2<u>C</u>H_2C=O), 28.63 (3 CH_3 of *t*Bu and <u>C</u>H_2CH_2C=O), 27.31 (CH_3 of *i*Pr), 25.29 (CH_3 of *i*Pr), 14.37 (CH_2<u>C</u>H_3). *IR* ν (cm^{-1}) = 3376, 2980, 2936, 1752, 1720, 1684, 1524, 1455, 1367, 1303, 1254, 1215, 1160, 1116, 1060, 1033, 984, 916, 883, 862, 796. *GC-MS* (initial temp. 70 °C): R_t 10.66 min: m/z 396 (M+-15, 0.8%), 311 (5.7), 254 (5.2), 198 (9.3), 197 (6.4), 181 (5.5), 172 (6.8), 171 (9.1), 170 (11), 154 (8.9), 153 (5.1), 152 (9.7), 151 (7.5), 143 (5.5), 131 (9.7), 130 (21), 129 (12), 126 (11), 125 (15), 113 (6.1), 112 (29), 108 (6.0), 101 (7.0), 97 (18), 85 (8.6), 84 (41), 83 (81), 82 (5.4), 81 (11), 69 (6.0), 59 (14), 58 (36), 57 (54), 56 (9.6), 55 (100), 54 (6.8), 53 (6.0), 43 (26), 42 (6.3), 41 (26), 39 (13). *HRMS* (ESI+): m/z calcd for $C_{21}H_{33}NO_7$ $[M+Na]^+$: 433.2155; found: 433.2150.



Ethyl (E)-3-((4R,5R)-5-(1-acetoxy-2-((2,6-dimethyl phenyl)amino)-2-oxoethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)acrylate 3.30

Prepared from crude aldehyde **3.22** (0.534 mmol), acetic acid (34 μL, 0.59 mmol), dimethylphenyl isocyanide (77 mg, 0.59 mmol) and $ZnBr_2$ (48 mg, 0.21 mmol) according to procedure B. At the end, the crude mixture was acetylated with Ac_2O (47 μL, 0.493 mmol, 1.2 eq.), Et_3N (114 μL, 0.82 mmol, 2 eq.) and 4-dimethylaminopyridine (10 mg, 0.08 mmol, 0.2 eq.). Analysis of the crude product by ^1H NMR revealed a d.r. of 91:9. The crude product was purified by column chromatography (ETP:DCM:Et_2O 3:1:1) affording diastereomerically pure **3.30_*anti*** (104 mg, 46%). On the basis of the determined d.r. we calculated an overall yield of **3.30_*anti*** + **3.30_*syn*** of 51%.

3.30_*anti* white solid; R_f = (0.37, ETP:DCM:Et_2O 3:2:2) developed with Hanessian stain. $[\alpha]^{20}_D$= - 48.78 (c = 0.98, $CHCl_3$). m.p. 123.8 – 127.1 °C (CH_2Cl_2).
^1H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 7.29 (broad s, 1H, NH), 7.13 – 7.01 (m, 3H, 3 CH Ar), 6.93 (dd, J = 15.6, 5.0 Hz, 1H, CHC<u>H</u>=CH), 6.18 (dd, J = 15.6, 1.7 Hz, 1H, O=CC<u>H</u>=CH), 4.96 (ddd, J = 6.5, 5.0, 1.7 Hz, 1H, CHC<u>H</u>O), 4.86 (d, J = 9.6 Hz, 1H, C<u>H</u>OAc), 4.70 (dd, J = 9.5, 6.2 Hz, 1H, C<u>H</u>CHOC=O), 4.20 (q, J = 7.1 Hz, 2H, C<u>H$_2$</u>CH$_3$), 2.20 (s, 6H, 2 CH_3 of 2.6-dimethylphenyl), 2.12 (s, 3H, CH_3 of OAc), 1.58 (s, 3H, CH_3 of *i*Pr), 1.42 (s, 3H, CH_3 of *i*Pr), 1.29 (t, J = 7.1 Hz, 3H, CH$_2$C<u>H$_3$</u>) *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 169.70 (C=ONH), 165.87 (C=O), 165.65 (C=O), 140.98 (CH<u>C</u>H=CH), 135.58 (2 C quat. Ar), 133.15 (C quat. Ar), 128.28 (2 CH Ar), 127.57 (CH Ar), 122.59 (O=C<u>C</u>H=CH), 110.27 (<u>C</u>(CH$_3$)$_2$), 76.37 (<u>C</u>HCHOAc), 76.08 (CH<u>C</u>HO), 71.38 (<u>C</u>HOAc), 60.77 (<u>C</u>H$_2$CH$_3$), 27.91 (CH_3 of *i*Pr), 25.26 (CH_3 of *i*Pr), 20.42 (CH_3 of OAc), 18.45 (2 CH_3 of 2.6-dimethylphenyl), 14.33 (CH$_2$<u>C</u>H$_3$). *IR* ν (cm^{-1}) = 3239, 2985, 2928, 2855, 1747, 1717, 1660, 1540, 1472, 1371, 1304, 1259, 1227, 1161, 1120, 1069, 1041, 982, 928, 880, 800, 766, 708, 685. *GC-MS* (initial temp. 70 °C): R_t 12.64 min: m/z 419 (M+-15, 8.7%), 419 (8.7), 404 (8.3), 361 (11), 219 (30), 199 (5.8), 190 (6.3), 179 (6.7), 178 (15), 177 (100), 176 (33), 172 (5.7), 160 (7.7), 148 (47), 147 (12), 143 (6.1), 126 (15), 125 (6.0), 122 (6.7), 121 (27), 120 (13), 112 (12), 109 (6.2), 106 (5.8), 105 (11), 101 (6.7), 97 (18), 91 (5.0), 85 (9.1), 84 (20), 81 (5.0), 77 (6.6), 59 (8.2), 55 (6.6), 43 (66), 39 (5.6). *HRMS* (ESI+): m/z calcd for $C_{22}H_{29}NO_7$ $[M+Na]^+$: 442.1842; found: 442.1846.

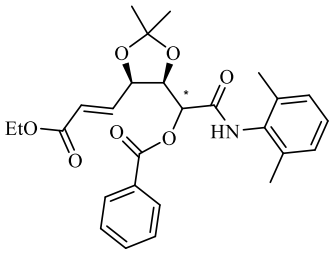


Ethyl (E)-3-((4R,5R)-5-(2-(cyclohexylamino)-1-(2-methoxyacetoxy)-2-oxoethyl)-2,2-dimethyl-1,3-dioxolan-4-yl) acrylate 3.31

Prepared from crude aldehyde **3.22** (0.61 mmol), 2-methoxyacetic acid (51 μL, 0.67 mmol), cyclohexyl isocyanide (83 μL, 0.67 mmol) and $ZnBr_2$ (55 mg, 0.24 mmol) according to procedure B. The crude product was purified by column

chromatography (ETP:DCM:Et_2O 3:2:2) affording **3.31_*anti*** and **3.31_*syn*** (d.r. = 84 : 18 calculated by ^1H NMR analysis on the crude product) (149 mg, 57%) as a mixture of diastereoisomers.

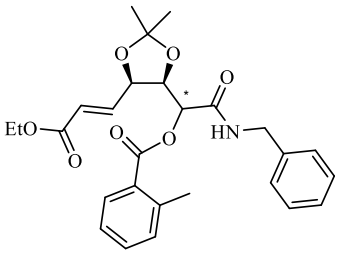
3.31_*anti* white solid; R_f = (0.20, ETP:DCM:Et_2O 3:2:2) developed with Hanessian stain. $[\alpha]^{20}_D$= - 43.13 (c = 1.03, $CHCl_3$). m.p. 146.2 – 148.7 °C (CH_2Cl_2).
^1H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 6.86 (dd, *J* = 15.6, 5.4 Hz, 1H, CHC<u>H</u>=CH), 6.12 (dd, *J* = 15.6, 1.6 Hz, 1H, CHC<u>H</u>=CH), 5.91 (d, *J* = 8.4 Hz, 1H, NH), 4.98 (d, *J* = 8.0 Hz, 1H, C<u>H</u>C=O), 4.88 (ddd, *J* = 6.9, 5.4, 1.6 Hz, 1H, CHC<u>H</u>O), 4.69 (dd, *J* = 8.0, 6.4 Hz, 1H, C<u>H</u>CHOC=O), 4.18 (q, *J* = 7.1 Hz, 2H, C<u>H_2</u>CH_3), 4.09 (d, *J* = 7.1 Hz, 2H, OC<u>H_2</u>C=O), 3.84 – 3.67 (m, 1H, C<u>H</u> of Cy), 3.44 (s, 3H, OC<u>H_3</u>), 1.93 – 1.80 (m, 2H, CH_2 of Cy), 1.75 – 1.53 (m, 4H, C<u>H_2</u>CH_3), 1.52 (s, 3H, CH_3 of *i*Pr), 1.39 (s, 3H, CH_3 of *i*Pr), 1.42 – 1.03 (m, 4H, CH_2 of Cy), 1.28 (t, *J* = 7.1 Hz, 3H, C<u>H_2</u>CH_3) . *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 169.02 (C=ONH), 165.81 (C=O), 165.55 (C=O), 141.12 (CH<u>C</u>H=CH), 122.70 (O=C<u>C</u>H=CH), 109.88 (<u>C</u>$(CH_3)_2$) , 76.27 (<u>C</u>HCHOC=O), 76.13 (CH<u>C</u>HO), 71.17 (<u>C</u>HOC=O), 69.40 (O<u>C</u>H_2C=O), 60.71 (<u>C</u>H_2CH_3), 59.50 (O<u>C</u>H_3), 48.42 (<u>C</u>H of Cy), 32.81 (<u>C</u>H_2 of Cy), 32.67 (<u>C</u>H_2 of Cy), 27.66 (<u>C</u>H_3 of *i*Pr), 25.51 (<u>C</u>H_2 of Cy), 25.08 (<u>C</u>H_3 of *i*Pr), 24.66 (2 <u>C</u>H_2 of Cy), 14.26 (CH_2<u>C</u>H_3). *IR* ν (cm^{-1}) = 3314, 2989, 2938, 2856, 1758, 1716, 1661, 1550, 1452, 1420, 1384, 1356, 1303, 1259, 1245, 1225, 1183, 1161, 1122, 1096, 1077, 1031, 978, 929, 889, 879, 853, 804, 760, 734, 720, 694, 665, 645. *GC-MS* (initial temp. 70 °C): R_t 12.39 min: m/z 412 (M+-15, 0.4 %), 170 (5.9), 168 (5.4), 154 (6.6), 152 (7.5), 130 (5.1), 125 (5.5), 112 (27), 109 (5.1), 98 (15), 97 (14), 85 (6.4), 84 (30), 83 (16), 81 (8.1), 59 (11), 56 (7.9), 55 (27), 45 (100), 43 (21), 41 (14), 39 (6.3). *HRMS* (ESI+): m/z calcd for $C_{21}H_{33}NO_8$ $[M+Na]^+$: 450.2104; found: 450.2101



2-((2,6-dimethylphenyl)amino)-1-((4R,5R)-5-((E)-3-ethoxy-3-oxoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxoethyl benzoate 3.33
Prepared from crude aldehyde **3.22** (0.65 mmol), benzoic acid (88 mg, 0.72 mmol) dimethylphenyl isocyanide (94 mg, 0.72 mmol) and $ZnBr_2$ (59 mg, 0.26 mmol) according to procedure B. Analysis of the crude product by HPLC revealed a d.r. of 89:11. The crude product was purified by column chromatography (ETP:DCM:Et_2O 3:1:1) affording diastereomerically pure **3.33_*anti*** (160 mg, 51%). On the basis of the determined d.r. we calculated an overall yield of **3.33_*anti*** + **3.33_*syn*** of 57%.

3.33_*anti* white solid; R_f = (0.31, ETP: Et_2O 4:6) developed with Hanessian stain. $[\alpha]^{20}_D$= + 25.16 (c = 1.03, $CHCl_3$). m.p. 152.7 – 153.2 °C (CH_2Cl_2).
^1H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 8.08 – 8.02 (m, 2H, 2 CH Ar.), 7.64 – 7.57 (m, 1H, 1 CH Ar.), 7.49 – 7.42 (m, 2H, 2 CH Ar.), 7.37 (s, 1H, NH), 7.13 – 7.03

(m, 3H, 3 CH Ar.), 6.96 (dd, *J* = 15.6, 5.4 Hz, 1H, CHC<u>H</u>=CH), 6.16 (dd, *J* = 15.6, 1.6 Hz, 1H, O=CC<u>H</u>=CH), 5.36 (d, *J* = 8.5 Hz, 1H, C<u>H</u>Bz), 5.06 – 5.00 (m, 1H, CHC<u>H</u>O), 4.94 (dd, *J* = 8.5, 6.4 Hz, 1H, C<u>H</u>CHOC=O), 3.97 (m, 2H, C<u>H</u>$_2$CH$_3$), 2.23 (s, 6H, 2 CH$_3$ of 2.6-dimethylphenyl), 1.58 (s, 3H, CH$_3$ of *i*Pr), 1.46 (s, 3H, CH$_3$ of *i*Pr), 1.08 (t, *J* = 7.1 Hz, 3H, C<u>H</u>$_2$CH$_3$). *^{13}C NMR* (75 MHz, CDCl$_3$, 25 °C, TMS): δ = 165.51 (2 C=O), 165.44 (C=O), 141.30 (CH<u>C</u>H=CH), 135.57 (2 C quat. Ar), 133.88 (CH Ar.), 133.10 (2 C quat. Ar), 130.09 (CH Ar.), 128.69 (2 CH Ar.), 128.34 (2 CH Ar.), 127.64 (2 CH Ar.), 123.42 (O=C<u>C</u>H=CH), 110.30 (<u>C</u>(CH$_3$)$_2$), 76.49 (<u>C</u>HCHOC=O and CH<u>C</u>HO), 71.84 (<u>C</u>HOC=O), 60.58 (<u>C</u>H$_2$CH$_3$), 27.89 (CH$_3$ of *i*Pr), 25.33 (CH$_3$ of *i*Pr), 18.56 (2 CH$_3$ of 2.6-dimethylphenyl), 14.10 (CH$_2$<u>C</u>H$_3$). *IR* ν (cm^{-1}) = 3247, 2987, 2935, 1726, 1671, 1602, 1515, 1475, 1452, 1371, 1263, 1223, 1176, 1161, 1110, 1071, 1030, 983, 880, 864, 766, 708, 682, 654, 619.

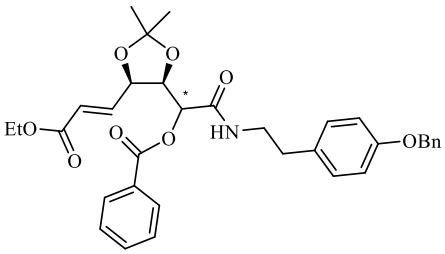


2-(benzylamino)-1-((4R,5R)-5-((E)-3-ethoxy-3-oxoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxoethyl 2-methylbenzoate 3.34

Prepared from crude aldehyde **3.22** (0.56 mmol), o-toluic acid (85 mg, 0.62 mmol), benzyl isocyanide (76 μL, 0.62 mmol) and ZnBr$_2$ (51 mg, 0.23 mmol) according to procedure B. Analysis of the crude product by ^{1}H NMR revealed a d.r. of 88:12. The crude product was purified by column chromatography (ETP:DCM:Et$_2$O 3:1:1) affording diastereomerically pure **3.34_*anti*** (134 mg, 49%). On the basis of the determined d.r. we calculated an overall yield of **3.34_*anti*** + **3.34_*syn*** of 56%.

3.34_*anti* white solid; R$_f$ = (0.69, ETP : Et$_2$O 4 : 6) developed with Hanessian stain. [α]$^{20}_D$= - 33.33 (c = 1.01, CHCl$_3$). m.p. 83.8 – 85.9 °C (CH$_2$Cl$_2$).
^{1}H NMR (300 MHz, CDCl$_3$, 25 °C,TMS): δ = 7.95 (dd, J = 8.2, 1.4 Hz, 1H, CH Ar), 7.43 (td, J = 7.7, 1.4 Hz, 1H, CH Ar), 7.35 – 7.21 (m, 7H, 7 CH Ar), 6.93 (dd, J = 15.6, 5.4 Hz, 1H, CHC<u>H</u>=CH), 6.46 (bt, J = 4.5 Hz, 1H, NH), 6.12 (dd, J = 15.6, 1.4 Hz, 1H, O=CC<u>H</u>=CH), 5.37 (d, J = 7.0 Hz, 1H, C<u>H</u>OC=O), 4.98 – 4.85 (m, 2H, CHC<u>H</u>O and C<u>H</u>CHOC=O), 4.61 and 4.33 (part AB of ABX syst., J$_{AB}$ = 15.0, J$_{AX}$ = 6.61, J$_{BX}$ = 5.09 Hz, 2 H, C<u>H</u>$_2$NH), 4.03 (qd, J = 7.1, 2.9 Hz, 2H, C<u>H</u>$_2$CH$_3$), 2.57 (s, 3H, C<u>H</u>$_3$C), 1.46 (s, 3H, CH$_3$ of *i*Pr), 1.41 (s, 3H, CH$_3$ of *i*Pr), 1.13 (t, J = 7.1 Hz, 3H, CH$_2$C<u>H</u>$_3$).
^{13}C NMR (75 MHz, CDCl$_3$, 25 °C, TMS): δ = 167.31 (C=ONH), 165.55 (C=O), 165.52 (C=O), 141.61 (CH<u>C</u>H=CH), 141.40 (C quat. Ar), 137.62 (C quat Ar), 132.86 (1 CH Ar), 131.92 (1 CH Ar), 130.91 (1 CH Ar), 128.71 (2 CH Ar), 127.74 (C quat. Ar), 127.64 (2 CH Ar), 127.59 (1 CH Ar), 125.95 (1 CH Ar), 123.33 (O=C<u>C</u>H=CH), 110.01 (<u>C</u>(CH$_3$)$_2$), 76.79 (<u>C</u>HCHOC=O), 76.29 (CH<u>C</u>HO), 71.40 (<u>C</u>HC=O), 60.54 (<u>C</u>H$_2$CH$_3$), 43.52 (<u>C</u>H$_2$NH), 27.42 (CH$_3$ of *i*Pr), 25.03 (CH$_3$ of *i*Pr), 21.82 (CH$_3$), 14.12 (CH$_2$<u>C</u>H$_3$).

IR ν (cm^{-1}) = 3331, 2984, 2931, 1723, 1664, 1601, 1551, 1494, 1455, 1434, 1387, 1367, 1307, 1235, 1208, 1173, 1157, 1141, 1071, 1029, 972, 922, 880, 858, 802, 737, 696, 668, 639.



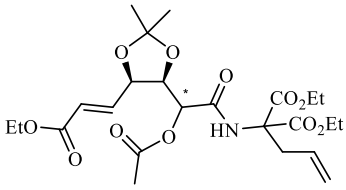
2-((4-(benzyloxy)phenethyl)amino)-1-((4R,5R)-5-((E)-3-ethoxy-3-oxoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxoethyl benzoate 3.36

Prepared from crude aldehyde **3.22** (0.61 mmol), benzoic acid (82 mg, 0.67 mmol), 1-(benzyloxy)-4-(2-isocyanoethyl)benzene (159 mg, 0.67 mmol) and $ZnBr_2$ (55 mg, 0.24 mmol) according to procedure B. Analysis of the crude product by ^{1}H NMR revealed a d.r. of 84:16.

The crude product was purified by column chromatography (ETP:DCM:Et_2O 3:1:1) affording diastereomerically pure **3.36_*anti*** (190 mg, 53%). On the basis of the determined d.r. we calculated an overall yield of **3.36_*anti*** + **3.36_*syn*** of 63%.

3.36_*anti* pale yellow solid; R_f = (0.25, ETP: Et_2O 4: 6) developed with Hanessian stain. $[\alpha]^{20}_D$= - 43.85 (c = 1.00, $CHCl_3$). m.p. 119.5 – 122.3 °C (CH_2Cl_2).

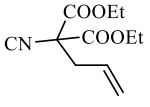
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 8.02 (d, J = 8.4 Hz, 2H, 2 CH Ar ortho of OBn), 7.60 (t, J = 7.4 Hz, 1H, CH Ar para of OBn), 7.46 (t, J = 7.7 Hz, 2H, CH Ar meta of OBn), 7.41 – 7.29 (m, 5H, 5 CH Ar of Bz), 7.04 (d, J = 8.4 Hz, 2H, 2 CH Ar ortho to OBn), 6.95 (dd, J = 15.2, 4.4 Hz, 1H, CHC<u>H</u>=CH), 6.76 (d, J = 8.3 Hz, 2H, 2 CH Ar meta to OBn), 6.19 – 6.15 (m, 1H, NH), 6.12 (d, J = 15.6 Hz, 1H, O=CC<u>H</u>=CH), 5.49 (d, J = 5.1 Hz, 1H, C<u>H</u>Bz), 4.99 – 4.87 (m, 4H, CHC<u>H</u>O, C<u>H</u>CHBz and OC<u>H$_2$</u>Ph), 4.03 (qq, J = 6.4, 3.6 Hz, 2H, C<u>H$_2$</u>CH_3), 3.47 (ddp, J = 19.9, 13.1, 6.7 Hz, 2H, C<u>H$_2$</u>NH), 2.72 (t, J = 6.9 Hz, 2H, C<u>H$_2$</u>CH_2NH), 1.37 (s, 6H, 2 CH_3 of *i*Pr), 1.13 (t, J = 6.9 Hz, 3H, CH_2C<u>H$_3$</u>). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 166.88 (C=ONH), 165.64 (C=O), 165.02 (C=O), 157.56 (C quat. Ar), 142.02 (CH<u>C</u>H=CH), 137.10 (C quat. Ar), 133.80 (CH Ar para of OBn), 130.74 (C quat. of Bz), 130.06 (2 CH Ar ortho of OBn) , 129.74 (2 CH Ar ortho to OBn), 128.76 (C quat. Ar), 128.68 (2 CH Ar meta of OBn), 128.65 (2 CH Ar of Bz), 128.02 (CH Ar of Bz), 127.47 (2 CH Ar of Bz), 123.17 (O=C<u>C</u>H=CH), 115.05 (2 CH Ar meta to OBn) , 109.85 (<u>C</u>(CH_3)$_2$), 76.95 (<u>C</u>HCHOC=O), 76.18 (CH<u>C</u>HO), 71.65 (<u>C</u>HBz), 69.99 (O<u>C</u>H_2Ph), 60.55 (<u>C</u>H_2CH_3), 40.67 (<u>C</u>H_2NH), 34.47 (<u>C</u>H_2CH_2NH), 27.29 (CH_3 of *i*Pr), 24.97 (CH_3 of *i*Pr), 14.17 (CH_2<u>C</u>H_3). *IR* ν (cm^{-1}) = 3409, 2983, 2937, 1714, 1683, 1611, 1583, 1530, 1511, 1453, 1379, 1241, 1177, 1162, 1111, 1070, 1026, 991, 878, 858, 806, 736, 711, 695. *HRMS* (ESI+): m/z calcd for $C_{34}H_{37}NO_8$ $[M+Na]^+$: 610.2417; found: 610.2427.



Diethyl 2-(2-acetoxy-2-((4R,5R)-5-((E)-3-ethoxy-3-oxoprop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)acetamido)-2-allylmalonate 3.32

Prepared from crude aldehyde **3.22** (0.53 mmol), acetic acid (34 μL, 0.59 mmol), diethyl 2-allyl-2-isocyanomalonate 3.48 (132 mg, 0.59 mmol) and $ZnBr_2$ (48 mg, 0.21 mmol) according to procedure B. At the end, the crude mixture was acetylated with Ac_2O (41 μL, 0.42 mmol, 1.2 eq.), Et_3N (97 μL, 0.71 mmol, 2 eq.) and 4-dimethylaminopyridine (9 mg, 0.07 mmol, 0.2 eq.). Analysis of the crude product by ^1H NMR and HPLC revealed a d.r. of 96:3. The crude product was purified by column chromatography (ETP:DCM:Et_2O 3:1:1) affording diastereomerically pure **3.32_*anti*** (167 mg, 61%). On the basis of the determined d.r. we calculated an overall yield of **3.32_*anti*** + **3.32_*syn*** of 64%.

3.32_*anti* pale yellow oil; R_f = (0.45, ETP:DCM:Et_2O 3:1:1) developed with Hanessian stain. $[\alpha]^{20}_D$= - 39.19 (c = 1.04, $CHCl_3$).
^1H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 7.44 (broad s, 1H, NH), 6.89 (dd, *J* = 15.6, 4.9 Hz, 1H, CHC<u>H</u>=CH), 6.15 (dd, *J* = 15.6, 1.3 Hz, 1H, O=CC<u>H</u>=CH), 5.68 – 5.49 (m, 1H, C<u>H</u>=CH_2), 5.15 – 5.06 (m, 2H, CH=C<u>H_2</u>) 4.92 – 4.88 (m, 1H, CHC<u>H</u>O), 4.86 (d, *J* = 9.2 Hz, 1H, C<u>H</u>OAc), 4.55 (dd, *J* = 9.2, 6.1 Hz, 1H, C<u>H</u>CHOC=O), 4.30 – 4.15 (m, 6H, 3 C<u>H_2</u>CH_3), 3.05 (dt, *J* = 10.5, 5.1 Hz, 2H, C<u>H_2</u>CH=CH_2), 2.09 (s, 3H, CH_3 of OAc), 1.60 (s, 3H, CH_3 of *i*Pr), 1.41 (s, 3H, CH_3 of *i*Pr), 1.31 – 1.21 (m, 9H, 3 CH_2C<u>H_3</u>). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 169.03 (C=ONH), 167.27 (C=O), 167.22 (C=O), 165.95 (C=O), 165.87 (C=O), 140.63 (CH<u>C</u>H=CH), 131.15 (<u>C</u>H=CH_2), 122.38 (O=C<u>C</u>H=CH), 120.04 (CH=<u>C</u>H_2), 110.27 (<u>C</u>$(CH_3)_2$), 76.39 (<u>C</u>HCHOAc), 75.98 (CH<u>C</u>HO), 70.28 (<u>C</u>HOAc), 66.31 (<u>C</u>$(COOEt)_2$), 62.71 (2 <u>C</u>H_2CH_3), 60.70 (<u>C</u>H_2CH_3), 36.92 (<u>C</u>H_2CH=CH_2), 27.87 (CH_3 of *i*Pr), 25.39 (CH_3 of *i*Pr), 20.40 (CH_3 of OAc), 14.30 (CH_2<u>C</u>H_3), 14.09 (CH_2<u>C</u>H_3), 14.06 (CH_2<u>C</u>H_3). *IR* ν (χm^{-1}) = 3410, 2984, 2925, 2855, 1740, 1720, 1695, 1505, 1466, 1370, 1305, 1259, 1213, 1178, 1160, 1096, 1060, 1014, 987, 928, 882, 857, 795, 756, 665. *GC-MS* (initial temp. 70 °C): R_t 12.29 min: m/z 498 (M+-15, 11%), 498 (11), 455 (9.6), 440 (11), 410 (5.8), 396 (5.9), 383 (6.7), 382 (35), 344 (15), 313 (7.5), 272 (5.5), 271 (19), 241 (10), 239 (8.9), 229 (7.7), 216 (7.5), 214 (10), 213 (6.3), 199 (34), 198 (13), 181 (12), 174 (13), 172 (6.0), 171 (25), 170 (27), 168 (7.1), 153 (12), 143 (20), 142 (51), 141 (9.1), 126 (7.3), 125 (17), 124 (10), 114 (5.4), 113 (8.5), 112 (58), 111 (6.0), 109 (6.6), 101 (14), 97 (37), 96 (16), 87 (6.7), 85 (17), 84 (41), 83 (7.1), 81 (7.5), 73 (5.2), 71 (5.8), 69 (11), 68 (26), 59 (12), 55 (9.4), 44 (8.6), 43 (100), 41 (14), 39 (6.9).

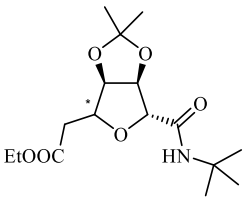


Diethyl 2-allyl-2-isocyanomalonate 3.49

A solution of diethyl 2-allyl-2-formamidomalonate (300 mg, 1.23 mmol) in dry CH_2Cl_2 (6 ml) was cooled to -30°C under argon atmosphere and treated with Et_3N (857 μL, 6.16 mmol), $POCl_3$ (169 μL, 1.85 mmol). After 1 h the

reaction was quenched with NaHCO3 (40 ml). The mixture was allowed to warm to room temperature and then extracted with Et2O. The crude product was purified by chromatography (ETP: AcOEt 8:2) to afford the product as a colourless oil (236 mg, 85%). The analytical data conform to those reported in literature[16b].

3.4.2.3 Cyclization reactions



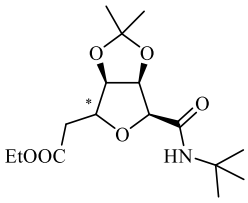
Ethyl 2-((3aR,6R,6aR)-6-(tert-butylcarbamoyl)-2,2-dimethyldihydro-4λ^3-furo[3,4-d][1,3]dioxol-4(3aH)-yl) acetate 3.37

Compound **3.23_*anti*** (100 mg, 0.23 mmol) was dissolved in dry ethanol (2.3 ml) under nitrogen atmosphere and sodium ethoxide (1.6 mL, 0.1 M, prepared in situ from Na and EtOH) was added. After 5 h the reaction mixture was poured into NH_4Cl (10 mL) and extracted with dichloromethane (3 x 10 ml). The organic phase was then washed with brine, dried (Na_2SO_4), and concentrated. Analysis of the crude product by ^{1}H NMR revealed a d.r. of 90:10. The residue was purified by chromatography (ETP: Et_2O 1:1) affording diastereomerically pure **3.37a** (50 mg, 66%). On the basis of the determined d.r. we calculated an overall yield of **3.37a** + **3.37b** of 73%.
Compounds **3.37a** and **3.37b** were also obtained under the same conditions starting from **3.29_*anti*.** Yield: 58%, d.r. 90:10 (calculated by ^{1}H NMR analysis on the crude product).

3.37a pale yellow solid; R_f = (0.48, ETP: Et_2O 1:1) developed with Hanessian stain. $[\alpha]^{21}_D$= - 2.94 (c = 1.00, $CHCl_3$). m.p. 61.1 – 62.4 °C (CH_2Cl_2).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 6.70 (s, 1H, NH), 5.17 (dd, *J* = 6.0, 0.7 Hz, 1H, C<u>H</u>CHC=O), 4.62 (dd, *J* = 6.0, 3.6 Hz, 1H, C<u>H</u>CHCH$_2$), 4.30 (s, 1H, C<u>H</u>C=O), 4.18 (qd, *J* = 7.1, 4.2 Hz, 2H, C<u>H$_2$</u>CH$_3$), 4.12 – 4.02 (m, 1H, CHC<u>H</u>CH$_2$), 2.81 and 2.70 (part AB of ABX syst., J_{AB} = 17.3, J_{AX} = 9.31, J_{BX} = 3.65 Hz, 2 H, CHC<u>H$_2$</u>C=O) 1.46 (s, 3H, CH$_3$ of *i*Pr), 1.36 (s, 9H, 3 CH$_3$ of *t*Bu), 1.31 (s, 3H, CH$_3$ of *i*Pr), 1.28 (t, *J* = 7.1 Hz, 3H, CH$_2$C<u>H$_3$</u>). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 171.24 (C=ONH), 168.05 (C=O), 112.85 (<u>C</u>(CH$_3$)$_2$), 84.05 (<u>C</u>HC=O) , 83.77 (<u>C</u>HCHC=O), 80.89 (<u>C</u>HCHCH$_2$), 77.74 (CH<u>C</u>HCH$_2$), 60.98 (<u>C</u>H$_2$CH$_3$), 51.18 (<u>C</u>(CH$_3$)$_3$), 33.95 (CH<u>C</u>H$_2$C=O), 28.81 (3 CH$_3$ of *t*Bu), 26.27 (CH$_3$ of *i*Pr), 25.14 (CH$_3$ of *i*Pr), 14.35 (CH$_2$<u>C</u>H$_3$). *IR* ν (cm^{-1}) = 3389, 2988, 2964, 2936, 1729, 1683, 1524, 1480, 1457, 1403, 1395, 1375, 1364, 1308, 1280, 1268, 1251, 1237, 1205, 1179, 1159, 1106, 1072, 1057, 1038, 1024, 992, 973, 927, 897, 866, 839, 825, 809, 763, 698, 648, 616. *GC-MS* (initial temp. 70 °C): R_t 9.14 min: m/z 314 (M+-15, 0.8%), 226 (14), 186 (18), 185 (12), 184 (100), 172 (17), 171 (30), 170 (16), 155 (20), 154 (5.4), 143 (8.5), 129 (9.3), 128 (26), 127 (14), 126 (27), 125 (11), 109 (5.1), 101 (27), 100 (6.3), 99 (11), 98 (14), 97 (18), 88 (18), 87 (30), 85 (41), 84 (17), 83 (7.8), 82 (7.0), 81 (37), 74 (7.0), 73 (7.7), 71 (11), 70 (8.2), 69 (8.6), 59 (18), 58 (33), 57 (79), 56 (7.1), 55 (15), 43 (39), 42 (9.0), 41 (32),

39 (7.0). *HRMS* (ESI+): m/z calcd for $C_{16}H_{27}NO_6$ $[M+Na]^+$: 352.1736; found: 352.1728.

3.37b colourless oil; R_f = (0.36, ETP: Et_2O 1:1) developed with Hanessian stain.
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 6.57 (s, 1H, NH), 4.94 (dd, *J* = 6.4, 2.8 Hz, 1H, C<u>H</u>CHC=O), 4.51 (dd, *J* = 6.3, 3.8 Hz, 1H, C<u>H</u>CHCH$_2$), 4.42 (dd, *J* = 7.9, 4.1 Hz, 1H, CHC<u>H</u>CH$_2$), 4.36 (d, *J* = 2.8 Hz, 1H, C<u>H</u>C=O), 4.18 (q, *J* = 7.2 Hz, 2H, C<u>H$_2$</u>CH$_3$), 2.66 and 2.54 (part AB of ABX syst., J_{AB} = 15.4, J_{AX} = 4.58, J_{BX} = 7.99. Hz, 2 H, CHC<u>H$_2$</u>C=O), 1.54 (s, 3H, CH$_3$ of *i*Pr), 1.36 (d, *J* = 2.3 Hz, 9H, 3 CH$_3$ of *t*Bu), 1.33 (s, 3H, CH$_3$ of *i*Pr), 1.28 (t, *J* = 7.1 Hz, 3H, CH$_2$C<u>H$_3$</u>).*^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 170.33 (C=ONH), 169.22 (C=O), 114.27 (<u>C</u>(CH$_3$)$_2$), 84.63 (<u>C</u>HC=O), 83.98 (<u>C</u>HCHC=O), 83.57 (<u>C</u>HCHCH$_2$), 82.42 (CH<u>C</u>HCH$_2$), 61.10 (<u>C</u>H$_2$CH$_3$), 51.28 (<u>C</u>(CH$_3$)$_3$), 38.14 (CH<u>C</u>H$_2$C=O), 28.77 (3 CH$_3$ of *t*Bu), 27.31 (CH$_3$ of *i*Pr), 25.47 (CH$_3$ of *i*Pr), 14.36 (CH$_2$<u>C</u>H$_3$). *GC-MS* (initial temp. 70 °C): R_t 9.40 min: m/z 314 (M+-15, 0.7%), 271 (16), 254 (6.3), 229 (17), 215 (32), 198 (7.7), 184 (16), 173 (5.2), 172 (46), 171 (21), 170 (9.3), 155 (11), 152 (13), 143 (11), 135 (5.4), 129 (7.2), 128 (6.4), 127 (14), 126 (19), 125 (14), 109 (9.9), 101 (18), 100 (6.0), 99 (8.8), 98 (10), 97 (32), 88 (33), 86 (6.7), 85 (100), 84 (17), 83 (7.7), 81 (18), 74 (6.5), 73 (7.8), 71 (12), 70 (9.9), 69 (10), 61 (6.8), 60 (6.2), 59 (24), 58 (35), 57 (82), 56 (8.3), 55 (15), 43 (52), 42 (13), 41 (40), 39 (9.5).

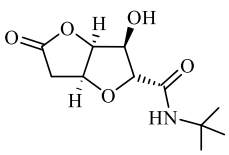


Ethyl 2-((3aR,6S,6aR)-6-(tert-butylcarbamoyl)-2,2-dimethyldihydro-4λ^3-furo[3,4-d][1,3]dioxol-4(3aH)-yl) acetate 3.38

Prepared using the same procedure described for **3.37**, starting from **3.23*_syn*** (63 mg, 0.17 mmol). Analysis of the crude product by ^{1}H NMR revealed a diastereomeric ratio of 84:16. Column chromatography on silica gel (ETP/Et_2O 3:7) gave **3.38a** and **3.38b** as an inseparable diastereomeric mixture (as a pale yellow oil) in 73% yield. Compounds **3.38a** and **3.38b** were also obtained under the same conditions starting from **3.29*_syn***. Yield: 65%, d.r. 83:17 (calculated by ^{1}H NMR analysis on the crude product).

3.38a/3.38 b: R_f = (0.34, ETP: Et_2O 1:1, single TLC spot) developed with Hanessian stain.
Legend for NMR spectra: M= major diastereoisomer, m= minor diastereoisomer
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS) mixture of the two diasteroisomers (M + m) not separable: δ = 6.35 (s, 1H, NH, m), 6.23 (s, 1H, NH, M), 5.02 (dd, *J* = 5.5, 4.4 Hz, 1H, C<u>H</u>CHC=O, m), 4.97 (dd, *J* = 6.0, 4.2 Hz, 1H, C<u>H</u>CHC=O, M), 4.72 (dd, *J* = 6.0, 3.6 Hz, 1H, C<u>H</u>CHCH$_2$, M), 4.66 – 4.57 (m, 2H, C<u>H</u>CHCH$_2$ and CHC<u>H</u>CH$_2$, m), 4.28 (d, *J* = 4.3 Hz, 1H, C<u>H</u>C=O, m), 4.24 – 4.13 (m, 4H, C<u>H$_2$</u>CH$_3$, M and m), 4.04 (dd, *J* = 6.7, 3.6 Hz, 1H, CHC<u>H</u>CH$_2$, M), 4.01 (d, *J* = 4.2 Hz, 1H, C<u>H</u>C=O, M), 2.83 and 2.78 (part AB of ABX syst., J_{AB} = 15.22, J_{AX} = 3.46, J_{BX} = 3.83. Hz, 2 H, CHC<u>H$_2$</u>C=O, M),

2.50 and 2.45 (part AB of ABX syst., J_{AB} = 14.37, J_{AX} = 5.14, J_{BX} = 4.19 Hz, 2 H, CHC$\underline{H_2}$C=O, m), 1.46 (s, 3H, CH_3 of *i*Pr, m), 1.43 (s, 3H, CH_3 of *i*Pr, M), 1.37 (s, 18H, CH_3 of *t*Bu, M and m), 1.31 – 1.26 (m, 12H, CH_3 of *i*Pr (M and m) and $CH_2C\underline{H_3}$ (M and m)). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS) mixture of the two diasteroisomers (M + m) not separable: δ = 170.82 (C=ONH, M), 170.19 (C=ONH, m), 166.72 (OC=O, m) 166.48 (OC=O, M), 113.02 ($\underline{C}(CH_3)_2$, m), 112.72 ($\underline{C}(CH_3)_2$, M), 84.09 ($\underline{C}HCHCH_2$, m), 81.93 ($\underline{C}HC$=O, M), 81.71 ($\underline{C}HCHC$=O, M and m), 81.33 ($CH\underline{C}HCH_2$ and $\underline{C}HC$=O, m), 80.45 ($\underline{C}HCHCH_2$, M), 77.83 ($CH\underline{C}HCH_2$, M), 60.94 ($\underline{C}H_2CH_3$, M), 60.54 ($\underline{C}H_2CH_3$, m), 51.28 ($\underline{C}(CH_3)_3$, M and m), 36.40 ($CH\underline{C}H_2C$=O, m), 33.89($CH\underline{C}H_2C$=O, M), 28.96 (3 CH_3 of *t*Bu, M and m), 26.34 (CH_3 of *i*Pr, m), 25.99 (CH_3 of *i*Pr, M), 24.65 (CH_3 of *i*Pr, M), 24.58 (CH_3 of *i*Pr, m), 14.31($CH_2\underline{C}H_3$, M and m). *IR* mixture of the two diasteroisomers (M + m) not separable ν (cm^{-1}) = 3417, 2976, 2936, 2908, 2876, 1734, 1681, 1525, 1456, 1366, 1332, 1288, 1266, 1229, 1209, 1181, 1163, 1101, 1045, 1028, 983, 951, 921, 895, 861, 800, 723. *GC-MS* (initial temp. 70 °C): Rt_1 = 9.33 min: m/z 314 (M+ -15, 21.0%), 314 (21), 284 (15), 229 (6.1), 228 (5.5), 184 (9.3), 173 (8.4), 172 (100), 171 (14), 170 (9.3), 155 (16), 154 (11), 152 (10), 143 (6.8), 128 (10), 127 (11), 126 (55), 125 (7.8), 101 (15), 100 (6.0), 99 (11), 98 (22), 97 (11), 88 (12), 87 (22), 85 (26), 84 (13), 83 (6.4), 82 (5.7), 81 (29), 71 (7.2), 70 (5.2), 59 (12), 58 (22), 57 (30), 55 (7.8), 43 (18), 41 (14); Rt_2 = 9.42 min: m/z 314 (M+ -15, 7.1%), 314 (7.1), 284 (5.2), 242 (5.8), 229 (15), 215 (16), 212 (8.0), 186 (6.6), 184 (14), 173 (5.7), 172 (47), 171 (69), 170 (18), 155 (15), 154 (16), 146 (8.0), 143 (11), 129 (6.1), 128 (8.1), 127 (11), 126 (29), 125 (29), 115 (5.8), 101 (17), 100 (6.1), 99 (11), 98 (16), 97 (31), 89 (6.7), 88 (47), 87 (5.2), 86 (7.2), 85 (100), 84 (28), 83 (8.2), 82 (6.6), 81 (34), 73 (8.2), 72 (6.0), 71 (14), 70 (13), 69 (11), 61 (7.6), 60 (6.3), 59 (31), 58 (41), 57 (63), 56 (8.4), 55 (15), 44 (5.3), 43 (47), 42 (13), 41 (35), 39 (7.7). *HRMS* (ESI+): m/z calcd for $C_{16}H_{27}NO_6$ $[M+Na]^+$: 352.1736; found: 352.1720.



(2R,3R,3aS,6aS)-N-(tert-butyl)-3-hydroxy-5-oxohexahydrofuro[3,2-b]furan-2-carboxamide 3.40

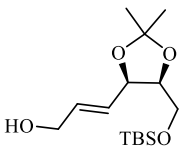
Compound **3.40** was synthetized using two different procedures.

Procedure 1[7a]

Compound **3.37a** (25 mg, 0.08 mmol) was dissolved in THF (759 μL) and water (379 μL) and treated with CF_3COOH (379 μL). The reaction was stirred at r.t. for 48 h and then at reflux for 6 days. The crude product was evaporated and chromatographed (AcOEt) to afford the pure product (17 mg, 92%) as a white solid.

Procedure 2[15]

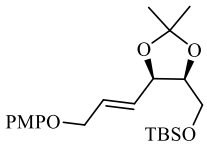
Compound **3.37a** (25 mg, 0.08 mmol) was dissolved in MeOH (173 μL) and water (108 μL) and treated with CF_3COOH (108 μL). The reaction was stirred at reflux for 3 days. The crude product was evaporated and chromatographed (AcOEt) to afford the pure product (16 mg, 87%) as a white solid.



(E)-3-((4R,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)prop-2-en-1-ol 3.44

Prepared as previously reported in literature[17].

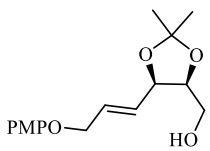
Colourless oil yield 96%.



Tert-butyl((4S,5R)-5-((E)-3-(4-methoxyphenoxy)prop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)dimethylsilane 3.45

Compound **3.44** (116 mg, 0.38 mmol), p-methoxyphenol (143 mg, 1.15 mmol), and triphenylphosphine (155 mg, 0.57 mmol) were dissolved in 3.8 mL of DCM dry under nitrogen atmosphere. Then diethyl azodicarboxylate (73 µL, 0.57 mmol) was added slowly at 0°C. The reaction mixture was then stirred for 4 d at room temperature. The reaction solvent was removed by evaporation under reduced pressure and the residue was purified by column chromatography on silica gel (ETP: Et_2O, 8:2) to afford product **3.45** (138 mg, 88%).

3.45 colourless oil R_f = (0.89, ETP: AcOEt 6:4) developed with Hanessian stain.
$[\alpha]^{23}_D$= + 2.46 (c = 0.95, $CHCl_3$).
^1H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 6.83 (s, 4H, 4 CH Ar), 6.01 (dt, *J* = 15.6, 4.9 Hz, 1H, CHC<u>H</u>=CH), 5.90 (dd, *J* = 15.6, 6.5 Hz, 1H, O=CC<u>H</u>=CH), 4.69 (t, *J* = 6.5 Hz, 1H, CHC<u>H</u>O), 4.49 (d, *J* = 4.9 Hz, 2H, C<u>H</u>$_2$OPMP), 4.20 (q, *J* = 6.1 Hz, 1H, C<u>H</u>CHOC=O), 3.76 (s, 3H, CH_3 of PMP), 3.60 (d, *J* = 6.0 Hz, 2H, C<u>H</u>$_2$OTBS), 1.47 (s, 3H, CH_3 of *i*Pr), 1.37 (s, 3H, CH_3 of *i*Pr), 0.88 (s, 9H, 3 CH_3 of *t*Bu), 0.05 (s, 3H, CH_3 of TBS), 0.04 (s, 3H, CH_3 of TBS). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 154.03 (C quat Ar), 152.87 (C quat Ar), 128.99 (CH<u>C</u>H=CH), 128.47 (O=C<u>C</u>H=CH), 115.79 (2CH Ar), 114.75 (2 CH Ar), 108.70 (<u>C</u>(CH$_3$)$_2$), 78.67 (<u>C</u>HCHOC=O), 77.84 (CH<u>C</u>HO), 68.50 (<u>C</u>H$_2$OPMP), 62.34 (<u>C</u>H$_2$OTBS), 55.84 (CH_3 of PMP), 27.96 (CH_3 of *i*Pr), 26.00 (3 CH_3 of *t*Bu), 25.49 (CH_3 of *i*Pr) , 18.38 (C(CH$_3$)$_3$), -5.28 (2 CH_3 of TBS). *IR* ν (cm^{-1}) = 2930, 2857, 1507, 1463, 1379, 1228, 1212, 1168, 1096, 1040, 972, 939, 834, 824, 775, 745, 714, 666. *GC-MS* (initial temp. 70 °C): R_t 11.58 min: m/z 408 (M+, 2.7%), 293 (20), 285 (6.0), 227 (5.4), 211 (12), 201 (11), 181 (19), 169 (17), 165 (9.6), 131 (9.3), 125 (16), 124 (37), 123 (37), 117 (8.4), 116 (9.9), 115 (11), 112 (8.0), 111 (100), 109 (8.0), 101 (7.2), 95 (25), 93 (6.0), 89 (29), 83 (5.9), 81 (6.0), 77 (8.8), 75 (39), 74 (8.7), 73 (90), 69 (52), 67 (8.6), 59 (25), 57 (7.8), 55 (8.4), 53 (18), 45 (6.1), 43 (31), 41 (34), 39 (5.8).

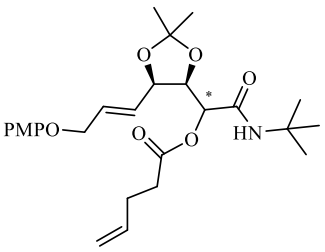


((4S,5R)-5-((E)-3-(4-methoxyphenoxy)prop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol 3.46

Compounds **3.46** was synthetized using the same procedure described for **3.3**, starting from **3.44** (203 mg, 0.49 mmol). After 24 h the reaction was quenched and the residue was chromatographed (ETP: Et_2O 3:7) affording the product as a colourless oil (133 mg, 92%).

3.46 colourless oil R_f = (0.03, ETP: Et_2O 7:3) developed with Hanessian stain.

$[\alpha]^{23}_D$= -33.74 (c = 0.74, $CHCl_3$).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 6.83 (s, 4H, 4 CH Ar of PMP), 6.04 (dt, *J* = 15.6, 5.1 Hz, 1H, CHC<u>H</u>=CH), 5.86 (dd, *J* = 15.6, 7.2 Hz, 1H, O=CC<u>H</u>=CH), 4.70 (t, *J* = 7.0 Hz, 1H, CHC<u>H</u>O), 4.51 (d, *J* = 5.0 Hz, 2H, C<u>H</u>$_2$OPMP), 4.26 (q, *J* = 5.7 Hz, 1H, C<u>H</u>CHOC=O), 3.76 (s, 3H, CH_3 of PMP) 3.55 (t, *J* = 5.6 Hz, 2H, CH_2OH), 1.86 (s, 1H, OH), 1.51 (s, 3H, CH_3 of *i*Pr), 1.39 (s, 3H, CH_3 of *i*Pr). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 154.16 (C quat. Ar), 152.62 (C quat. Ar), 130.24 (CH<u>C</u>H=CH), 127.77 (O=C<u>C</u>H=CH), 115.92 (2 CH Ar), 114.79 (2 CH Ar), 109.02 (<u>C</u>$(CH_3)_2$), 78.42 (<u>C</u>HCHOC=O), 77.39 (CH<u>C</u>HO), 68.28 (<u>C</u>H_2OPMP), 62.14 (<u>C</u>H_2OH), 55.86 (CH_3 of PMP), 27.91 (CH_3 of *i*Pr), 25.34 (CH_3 of *i*Pr). *IR* ν (cm^{-1}) = 3544, 3496, 2983, 2927, 2872, 1592, 1504, 1463, 1408, 1385, 1339, 1309, 1292, 1235, 1211, 1184, 1162, 1136, 1109, 1075, 1061, 1028, 1014, 977, 945, 897, 870, 851, 828, 800, 744, 649. *GC-MS* (initial temp. 70 °C): R_t 11.15 min: m/z 294 (M+, 2.6%), 125 (9.1), 124 (100), 123 (15), 111 (5.3), 109 (14), 95 (5.7), 81 (8.4), 69 (6.6), 59 (21), 57 (5.7), 55 (6.6), 53 (8.8), 44 (7.2), 43 (21), 41 (9.9). *HRMS* (ESI+): m/z calcd for $C_{16}H_{22}O_5$ $[M+Na]^+$: 317.1365; found: 317.1356.



2-(tert-butylamino)-1-((4R,5R)-5-((E)-3-(4-methoxy phenoxy)prop-1-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxoethyl pent-4-enoate 3.48

<u>Swern oxidation</u>

Aldehyde **3.47** was synthetized using the same procedure described for **3.22**, starting from 0.34 mmol of alcohol **3.46**.

<u>Passerini reaction</u>

Firstly, prepared from crude aldehyde **3.47** (0.34 mmol), pentenoic acid (38 μL, 0.37 mmol) and *tert*-butylisocyanide (42 μL, 0.37 mmol) according to procedure A. Analysis of the crude product by HPLC revealed a d.r. of 62:38. The residue was purified by column chromatography ((ETP:DCM:Et_2O 3:1:1) affording diastereomerically pure **3.48*_anti*** (66 mg, 41%). On the basis of the determined d.r. we calculated an overall yield of **3.48*_anti*** + **3.48*_syn*** of 64%.
Then, prepared from crude aldehyde **3.46** (0.34 mmol), pentenoic acid (38 μL, 0.37 mmol), *tert*-butylisocyanide (42 μL, 0.37 mmol) and $ZnBr_2$ (31 mg, 0.14 mmol) according to procedure B. Analysis of the crude product by HPLC revealed a d.r. of 77:23. The residue was purified by column chromatography ((ETP:DCM:Et_2O 3:1:1) affording diastereomerically pure **3.48*_anti*** (56 mg, 35%). On the basis of the determined d.r. we calculated an overall yield of **3.48*_anti*** + **3.48*_syn*** of 45%.

3.48*_anti*; colourless oil R_f = (0.03, ETP: Et_2O 7:3) developed with Hanessian stain.
$[\alpha]^{20}_D$= -22.70 (c = 0.45, $CHCl_3$).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = = 6.82 (s, 4H, 4 CH Ar of PMP), 6.02 (dt, J = 15.5, 4.8 Hz, 1H, CHC<u>H</u>=CH), 5.93 –5.82 (m, 2H, NH and O=CC<u>H</u>=CH), 5.82 – 5.70 (m, 1H, CH_2=C<u>H</u>CH_2) 5.08 – 4.93 (m, 3H, C<u>H</u>$_2$=CHCH_2 and C<u>H</u>OC=O), 4.77

(t, J = 6.3 Hz, 1H, CHC<u>H</u>O), 4.56 (dd, J = 7.6, 6.4 Hz, 1H, C<u>H</u>CHOC=O), 4.45 (d, J = 4.7 Hz, 2H, C<u>H$_2$</u>OPMP), 3.76 (s, 3H, CH_3 of PMP) 2.57 – 2.41 (m, 2H, $CH_2C\underline{H_2}C$=O), 2.41 – 2.30 (m, 2H, C<u>H$_2$</u>CH_2C=O) 1.50 (s, 3H, CH_3 of *i*Pr), 1.38 (s, 3H, CH_3 of *i*Pr), 1.33 (s, 9H, 3 CH_3 of *t*Bu). 13C NMR (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 171.63 (C=ONH), 166.38 (C=O), 154.13 (C quat. Ar), 152.72, (C quat. Ar) 136.49 (CH_2=<u>C</u>HCH_2), 129.46 (CH<u>C</u>H=CH), 127.11 (O=C<u>C</u>H=CH), 115.90 (<u>C</u>H_2=$CHCH_2$), 115.63 (2 CH Ar), 114.82 (2 CH Ar), 109.25 (<u>C</u>$(CH_3)_2$), 77.68 (<u>C</u>HCHOC=O), 76.50 (CH<u>C</u>HO), 71.65 (<u>C</u>HOC=O), 68.12 (CH_2OPMP), 55.86 (CH_3 of PMP), 51.70 (<u>C</u>$(CH_3)_3$), 33.29 ($CH_2\underline{C}H_2C$=O), 28.77 (3 CH_3 of *t*Bu), 28.57 (<u>C</u>H_2CH_2C=O), 27.77(CH_3 of *i*Pr), 25.20 (CH_3 of *i*Pr). *IR* ν (cm^{-1}) = 3391, 2977, 2935, 1745, 1689, 1507, 1455, 1367, 1216, 1163, 1107, 1063, 1035, 973, 916, 877, 824, 798, 744, 711. *GC-MS* (initial temp. 70 °C): R_t 13.03 min: m/z 475 (M+, 5.1%), 475 (5.1), 353 (21), 352 (100), 270 (13), 252 (11), 238 (5.8), 214 (14), 196 (9.2), 165 (5.1), 156 (7.9), 144 (8.8), 138 (14), 125 (8.1), 124 (54), 123 (20), 113 (5.5), 111 (33), 109 (11), 95 (13), 88 (11), 83 (31), 81 (7.1), 69 (8.9), 59 (7.9), 58 (17), 57 (32), 55 (54), 53 (13), 43 (13), 41 (15). *HRMS* (ESI+): m/z calcd for $C_{26}H_{37}NO_7$ $[M+Na]^+$: 498.2468; found: 498.2473.

3.5 Bibliography

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CHAPTER 4.
Application of biocatalysis and MCRs to the target-oriented synthesis of Bengamides

4.1 Target-oriented synthesis (TOS)

Target-oriented synthesis (TOS) is primarily used to access well-defined compounds, often complex natural products known for specific biological properties. Consequently, it addresses to a single point of chemical space.

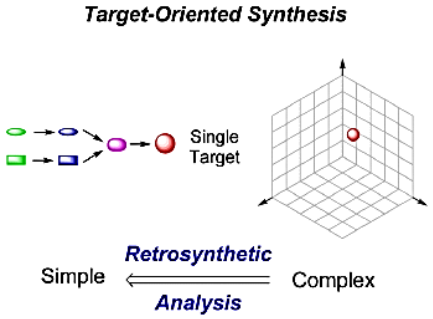
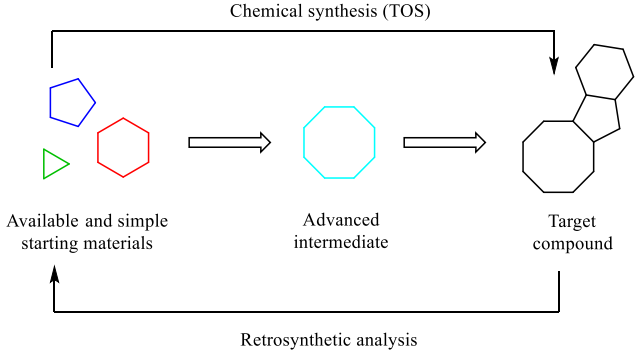


Figure 4.1 Target-oriented synthesis.
Taken from *Spandi et al.*[1]

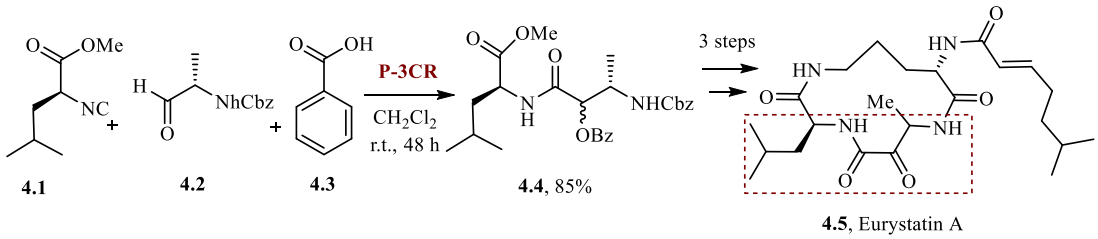
Synthetic pathways in TOS are linear and convergent, and they are planned by using retrosynthetic analysis (*Scheme 4.1*). E.J. Corey, who received the Nobel Prize in 1990 for this approach, defined retrosynthesis as the approach in which "*target structure is subjected to a deconstruction process which corresponds to the reverse of a synthetic reaction, so as to convert that target structure to simpler precursor structures, without any assumptions with regard to starting materials. Each of the precursors so generated is then examined in the same way, and the process is repeated until simple or commercially available structures result.*"[2]



Scheme 4.1 Schematic drawing of target-oriented synthesis and retrosynthetic analysis

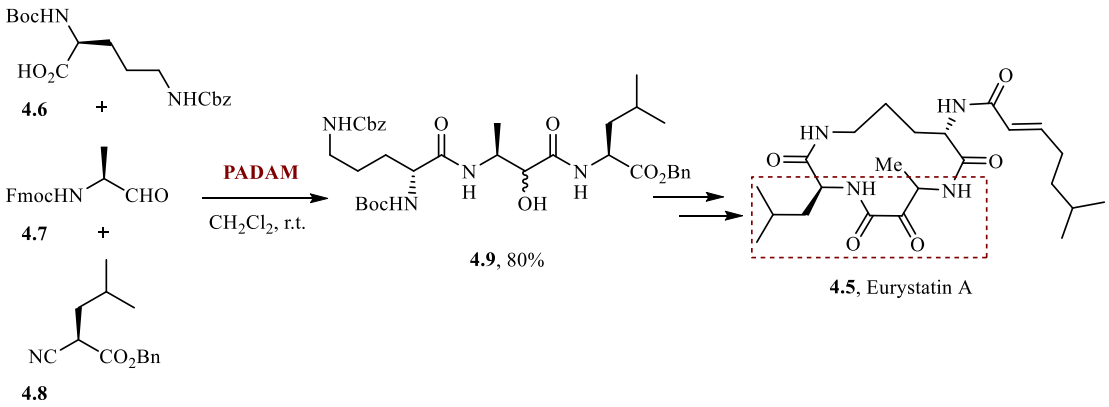
By saving synthetic operations while maximizing the build-up of structural and functional complexity, multicomponent reactions are particularly appealing in the context of target-oriented synthesis. Thanks to their numerous advantages (extensively described in *Chapter 2*), MCRs allow the synthesis of structurally complex substances more quickly and efficiently than the classical linear approach. However, although the use of multicomponent approach in total synthesis can be potentially very useful, its application, and in particular of IMCRs, in this field remained a quite unexplored area and "classic" approach based on the linear syntheses is still preferred. Some examples, reported in literature, of application of Passerini reaction (P-3CR) for the synthesis of bioactive compounds and natural products are described below.

Passerini reaction was used by Schmidt and collaborators[3] for the elaboration of a key fragment for the synthesis of the serine protease prolyl endopeptidase (PEP) inhibitor Eurystatin A **4.5** (*Scheme 4.2*). The reaction of methyl (S)-2-isocyano-4-methylpentanoate **4.1**, protected (S)- alanine **4.2** and benzoic acid **4.3**, under classical conditions, furnishes **4.4** as a diastereomeric mixture.



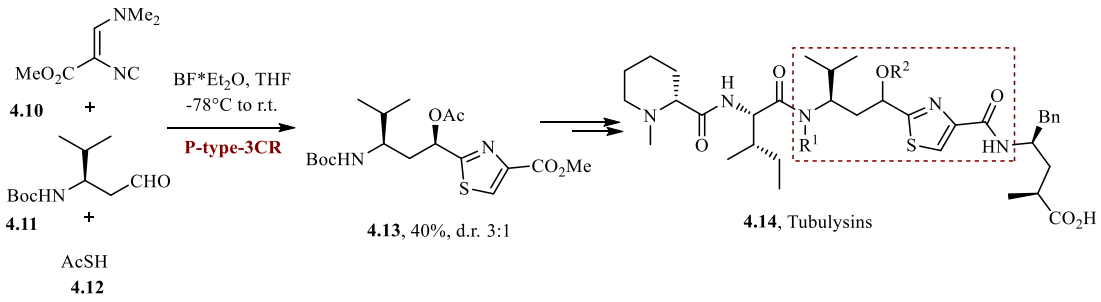
Scheme 4.2 Synthesis of Eurystatin A using Passerini reaction

In 2001 an improved synthesis of Eurystatin A was reported by Semple et al.[4]. who developed a highly efficient and atom-economic Passerini reaction/ deprotection/acyl migration (PADAM) strategy[5] for the rapid construction of the key intermediate **4.9** (*Scheme 4.3*). Just a few steps were required from there to complete the synthesis.



Scheme 4.3 Synthesis of Eurystatin A using a PADAM reaction

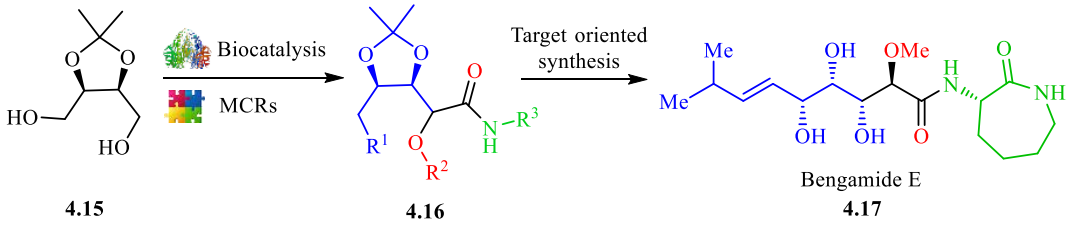
Another example of the application of Passerini reaction in target-oriented synthesis was reported by Domling and co-workers[6] (*Scheme 4.4*). They have developed a three-component Lewis acid-catalysed Passerini-type reaction for the synthesis of a precursor of Tubulysins **4.14**, a class of compounds that show promising in vivo anticancer properties and are candidates for antibody conjugates.



Scheme 4.4 Synthesis of Tubulysins by Lewis acid-catalysed Passerini-type reaction

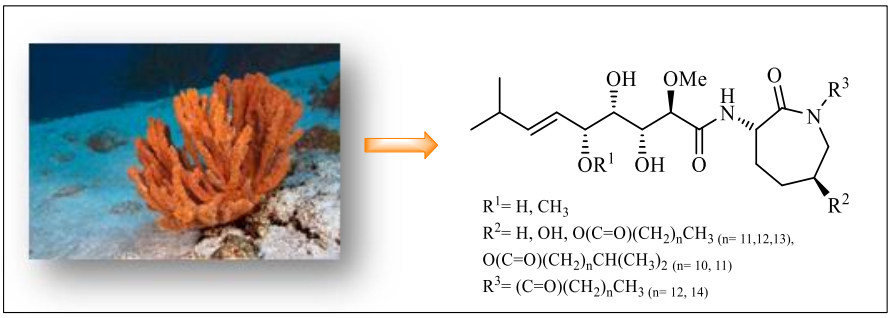
As illustrated by the examples above, Passerini reaction, and in general MCRs, represent an important tool for the synthesis of natural products, since they can afford the formation of several bonds in just one step with control of stereochemistry. Thus, comparing with linear synthesis, they can lead to complex molecules, such as natural products, though a convergent and shorter synthetic pathway.

In this context, my research project was based on the possibility of synthesizing Bengamide E, one of the members of a new class of antitumor natural products of marine origin, through a methodology that combines biocatalysis and Passerini reaction, starting from *meso* diol **4.15**.



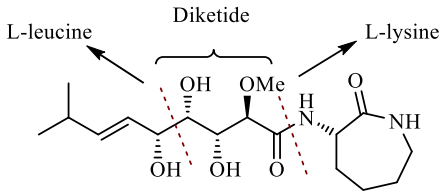
Scheme 4.5 Synthesis of Bengamide E starting from *meso* diol 4.15 using biocatalysis and multicomponent reaction.

Bengamides are a wide family of natural products of marine origin isolated by Crews and coworkers[7] between 1986 and 1989 from an undescribed specimen of an orange sponge belonging to the Jaspidae family. Until now 23 compounds of this family have been isolated and their structures elucidated.



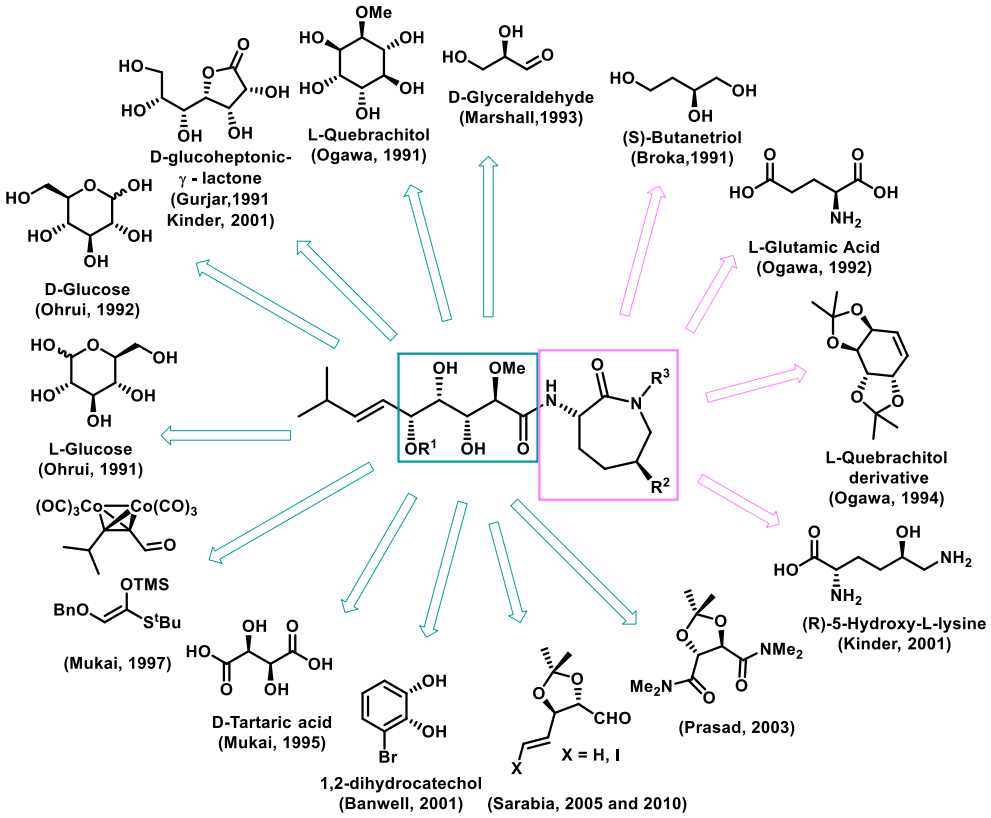
Scheme 4.6 Structure of Bengamides derived from *Jaspis cf. Coriacea* sponge

Several hypotheses on biosynthetic origin of Bengamides has been proposed; according to the most accepted[8] the C-10 backbone structure derives from the connection between a diketide and the L-leucine amino acid, on which subsequently the cyclized L- lysine is linked (*Scheme 4.7*).



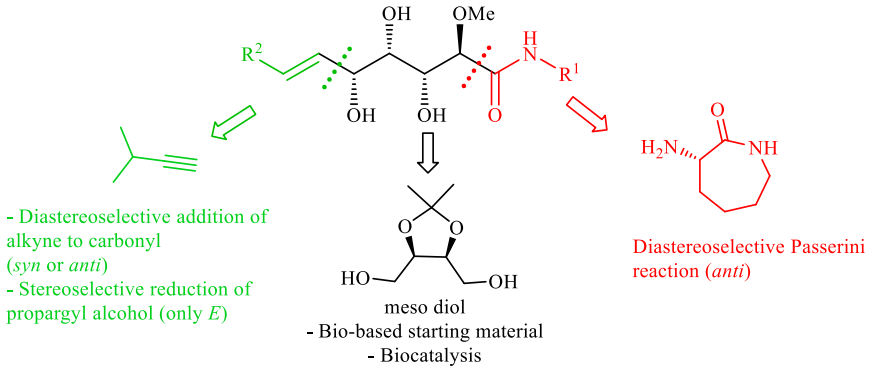
Scheme 4.7 Hypothesis for biosynthetic origin of Bengamides

Bengamides present a complex structure characterized by a chain with 10 carbon atoms with 4 contiguous stereocentres, a double bond with *E* configuration and a secondary amide which binds an aminocaprolactamic unit. Since they possess a promising biological profile as antibiotic, anthelmintic and antitumoral (as inhibitors of methionine aminopeptidases enzymes MetAP1 and MetAP2[9]), an intense research activity in the chemical and biological fields, including total syntheses and synthesis of analogues for biological evaluation has been carried out[10]. Some strategies for the synthesis of Bengamides and their analogues are illustrated in *Scheme 4.8*.



Scheme 4.8 Total synthesis of Bengamides and analogues in the period 1991-2010
Modified from *García-Ruiz and Sarabia*[10]

Through a retrosynthetic analysis, we developed a novel strategy for the total synthesis of Bengamides, and in particular of Bengamide E. Our idea is based on the possibility to synthesize the general structure of these compounds through the combination of three different fragments: the central skeleton derived from bio-based *meso* diol through biocatalysis, on which the olefinic residue is introduced through the formation of a new C-C bond, and on the other side the aminocaprolactamic unit is introduced thanks to a diastereoselective Passerini multicomponent reaction with a suitable isocyanide (*Scheme 4.9*).

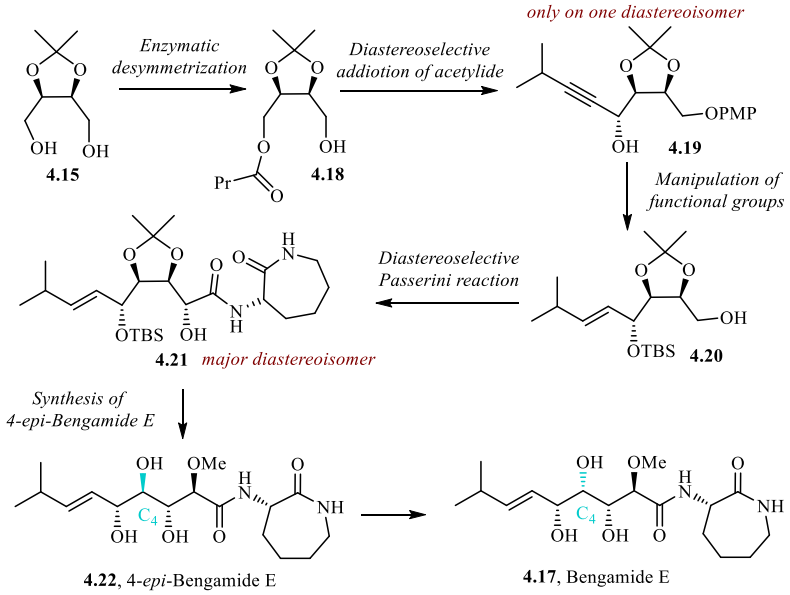


Scheme 4.9 Retrosynthetic analysis of Bengamide E

The main advantages of this strategy are the possibility to use a renewable source as starting material and the flexibility and convergence of the approach. In fact, Bengamide analogues can be easily obtained modifying caprolactamic unit and the olefinic residue by suitable use of different reagents. In addition, it also possible alter the different stereocentres of the molecule exploiting complementary enzymatic procedures to obtain the two *meso* diol derived enantiomers and performing the addition of acetylide and Passerini reaction with opposite diastereoselection. The possibility of achieving these modifications are of particular interest to the obtainment of more potent Bengamide derivatives.

4.2 Results and Discussion

This work was initiated before my doctoral research by master and bachelor students, whose work, after a thorough evaluation of different pathways, lead to the development of a synthetic strategy in 13 steps, (*Scheme 4.10* shows the key steps), based on the previously described approach.



Scheme 4.10 Synthetic strategy

The general strategy consists of four key steps:

1) Enantioselective generation of monoester **4.18** via biocatalysis.

2) Diastereoselective 1,2-additon of an acetylide, precursor of the alkenyl moiety. To obtain the desired diastereoisomer the reaction has to occur with an *anti*-selectivity.

3) Diastereoselective Passerini reaction using a chiral isocyanide to introduce the caprolactam moiety. The *anti*-selectivity is necessary as well.

4) Further elaboration of Passerini product to obtain 4-*epi*-Bengamide E **4.22**.

As shown in the scheme, to obtain Bengamide E **4.17** it would be necessary to add further synthetic steps to change the stereochemistry of one of the stereocentres (C4) of the polyhydroxylated chain. For this reason, initially, we planned to synthesize 4-*epi*-Bengamide E, which already presents the stereocentres obtained by the Passerini reaction.

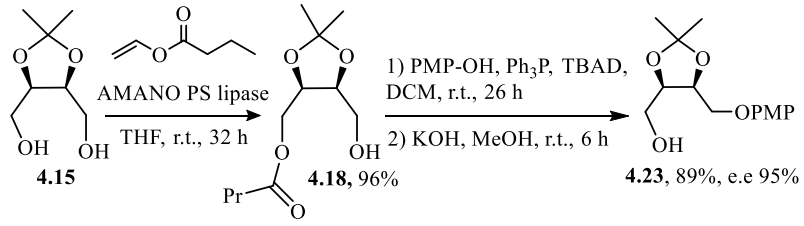
Due to presence of four contiguous stereocentres in the final product **4.22**, one of the main objectives of this synthetic sequence was the optimization of all the steps in order to obtain optically pure products and avoid the formation of complex mixture of diastereoisomers. With this aim, attention was devoted to the stereochemical issues of the addition of acetylide and of the Passerini reaction. In addition, the optical purity of the compounds was checked along the synthetic pathways. In fact, the presence of synthetic racemizing steps, mainly during the preparation of the building blocks for the Passerini reaction, could lead to the formation of products with low optical purity, causing waste of starting materials and time.

In the following paragraphs, some of the work previously done will be described and the results obtained during my research will be extensively discussed. In detail, after a brief optimization of some reaction conditions to obtain compound **4.20** from compound **4.18**, my work was mainly focused on the study of the last steps (from Passerini reaction to final product) of this strategy.

4.2.1 Enzymatic synthesis of chiral building blocks

As described in *Chapter 4*, diol **4.15** is a symmetrical achiral molecule. To afford the enantiomerically pure building block, we planned to apply a chemoenzymatic approach, involving an AMANO PS Lipase for the monoacylation of compounds **4.15** (*Scheme 4.11*). In a previous optimization, the use of vinyl butyrate had been preferred to vinyl acetate, due to the instability of the latter in the subsequent Mitsunobu reaction. In fact, a low e.e. (from 96% to 76%) had been observed due to the migration of the acetyl group and subsequent racemization. On the contrary, the use of butyrate group, probably due to the major steric hindrance, can avoid the migration process. The enzymatic reaction was performed following the procedure reported in literature[11], and, stopped at 56% conversion, allowing us to isolate the monoacylated compound **4.18** in good yield (96%). The enantiomeric excess (95%) was determined, in a later step, by HPLC analysis with a chiral column on product **4.23** that presents a chromophore necessary to make the molecule visible to the UV detector. Once obtain compound **4.18** the synthetic strategy involves the Mitsunobu and deacylation reactions to introduce the *p*-methoxyphenyl (PMP) group (*Scheme 4.11*). The change of functional group is necessary to obtain the desired monomer, that could also conveniently obtain by hydrolysis of the corresponding diester[11], but mainly because of the instability of the acyl group in the subsequent reactions, as a previous study had demonstrated. Thus, after a careful evaluation, PMP was chosen as

protecting group since it has orthogonally cleavage conditions (reaction with ammonium cerium (IV) nitrate) respect to the other protecting groups present on the molecule (acetonide and TBS group are removed under acidic conditions). In fact, it has to be removed in a previous step of the synthesis, as shown in *Scheme 4.10* and described in the next paragraph.



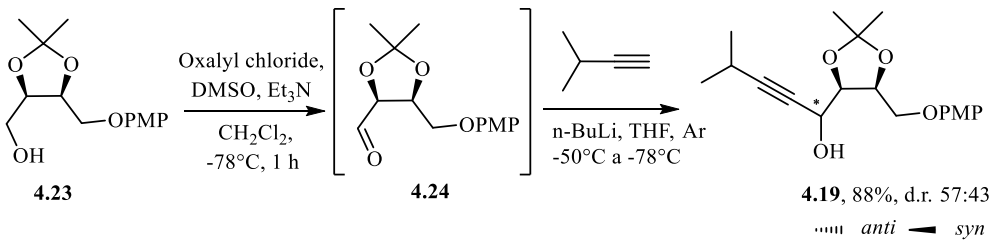
Scheme 4.11 Enzymatic desymmetrization and change of protecting group

Mitsunobu reaction was performed at room temperature in presence of *p*-methoxy phenol (PMP-OH), triphenylphosphine and di-*tert*-butyl azodicarboxylate (TBAD). The crude was directly subjected to the next reaction with KOH in MeOH to remove the acyl group. With these conditions product **4.23** was obtained in high yield (89%) also on a large scale (about 5 grams).

4.2.2 Diastereoselective addition of acetylide

In earlier work, some attempts to directly introduce the olefinic residue had been tried, using organolithium and Grignard reagents, but they didn't lead to good results. Therefore, we tried to perform the nucleophilic addition of acetylide to carbonyl compounds. This strategy leads to the formation of propargyl alcohol[12] which can be selectively reduced to double bond with the *E* configuration using aluminium hydrides. This method presents two advantages: acetylides can be easily obtained from corresponding terminal alkyne through a metalation reaction (lithium-hydrogen exchange) and many alkynes are commercially available

Alcohol **4.23** was oxidized under Swern conditions and the corresponding aldehyde was subjected to the 1,2- addition of the acetylide, generated in situ (*Scheme 4.12*).



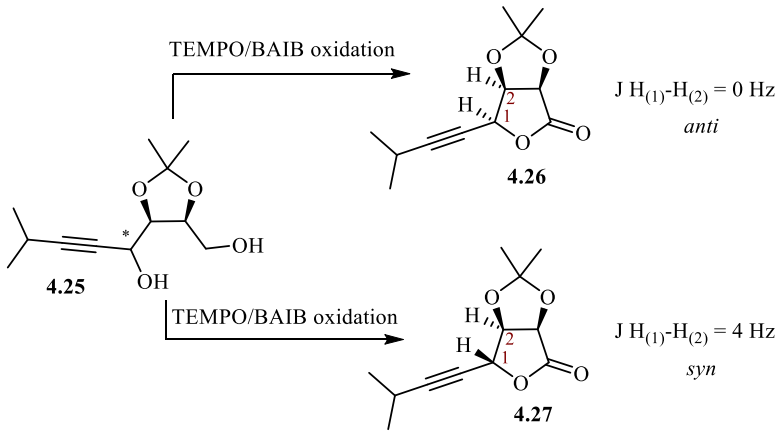
Scheme 4.12 Swern oxidation and 1,2 addition of acetylide to aldehyde

Among the different strategy to oxidize alcohols to aldehydes, Swern oxidation presents some important features that makes it particularly interesting: the reaction is generally fast (on this type of substrate it occurs in about an hour), the product is usually obtained in high yields and purification is not necessary since the by-products are all volatile. This last point represents a fundamental advantage in the presence of unstable aldehyde, as in our case. In fact, aldehyde **4.24** is unstable on silica and decomposes rapidly once it is dried. For this reason, it is important to carefully evaporate the solvent on the rotavapor without completely drying the crude product and to use it immediately after the work up without chromatographic purification. In addition, this aldehyde tends to remain in the hydrated form and, therefore, the presence of water within the reaction environment affects the success of the subsequent reaction.

Due to these problems, a thorough optimization of the work up conditions was accomplished to make the subsequent acetylide attack efficient and reproducible.

Different precautions were taken to make the aldehydes as dry as possible. Swern reaction was carried out under classical conditions; after work up the crude product was filtered through a sintered funnel filled with celite, dry Na_2SO_4 (dried in an oven), and celite layers in order to remove the water. Concerning the addition of acetylide, THF dried on molecular sieves, argon atmosphere and 4Å molecular sieves were employed during the reaction. Moreover, 2,2-bipyridyl was employed to control the efficiency of the *n*BuLi, alkyne and THF system. In this way, product **4.19** was obtained with a high yield (88%) but low diastereoselectivity (d.r. 57:43 *anti*:*syn*).

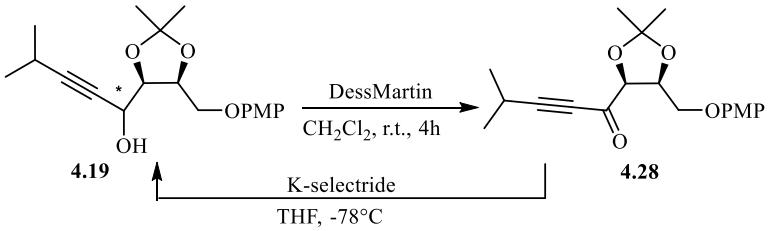
The absolute stereochemistry of propargyl alcohols has been determined, at an earlier stage of the research, by the formation of the corresponding lactones, after cleavage of the protecting group, and analysis of the J constant coupling by ^{1}H-NMR spectra (*Scheme 4.13*).



Scheme 4.13 Determination of stereochemistry of propargyl alcohol

Since only the *anti* diastereoisomer has the same configuration of the stereocentre adjacent to the triple bond of the 4-*epi*-Bengamide E, it is important to have a good diastereoselection to obtain a major quantity of the desired diastereoisomer. Consequently, in order to improve the d.r. of compound **4.19**, the diastereoisomeric mixture was oxidized to ketone with Dess Martin periodinane and the stereoselective reduction of the ketone was studied. Different reagents were used in a previous optimization *(Table 4.1)*.

Table 4.1 Optimization of diastereoselective reduction



Entry	*Reagent*	*Solvent*	*T (°C)*	*Time*	*d.r. (anti/syn)*[a]
1	Red-Al	THF	-78	1 h	49:51
2	L-selectride	THF	-78	1 h	63:37
3	$NaBH_4$	MeOH	-78	1 h	24:76
4	K-selectride	THF	-78	1 h	76:24
5	$MgBr_2{\cdot}Et_2O$ DIBAL-H	CH_2Cl_2/Et_2O 2:4	0 to rt	6 h	43:57
6	$MgBr_2{\cdot}Et_2O$ DIBAL-H	CH_2Cl_2/Et_2O 2:4	- 40 to rt	2 h	58:42

[a] Determined by HPLC

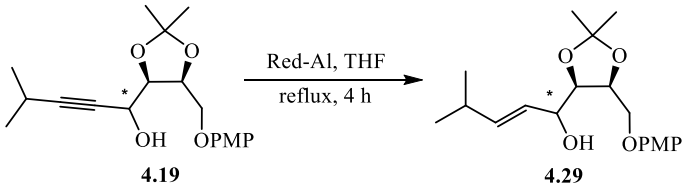
In all cases the reactions were performed until completion (conversion 100 % determined by HPLC) and the d.r. were determined by HPLC analysis. As shown in the table, using Red-Al, L-selectride and DIBAL-H in presence of $MgBr_2{\cdot}Et_2O$ as additive, a poor diastereoselection was observed (*Entries 1, 2, 5 and 6*); meanwhile, $NaBH_4$ afforded *syn* diastereoisomer. Best results were obtained with K- selectride (*Entry 4*, yield 72%, d.r.76:24, *anti: syn*).

4.2.3 Manipulation of functional groups

To obtain alcohol **4.20**, the substrate of Passerini reaction, it is necessary to reduce the triple to double bond and to introduce the TBS protecting group. The description of these steps follows in this paragraph.

Propargyl alcohols can conveniently be reduced to alkenes with sodium bis(2-methoxyethoxy)aluminium hydride (Red-Al) in dry THF. The preliminary results

obtained were not satisfactory; in fact, the major problems were the difficulty for the reaction to go to completion and the difficulty to separate the unreacted starting material from the product, ending up in an only moderate yield (67% after 17h). To solve this problem the reaction was performed at reflux rather than at room temperature, following a procedure reported in literature[13].



4.19 **4.29**

Scheme 4.14 Reduction of the triple bond

The reaction was initially carried out on the mixture of the two diastereoisomers of **4.19** to verify if they were more easily separable at the level of the double bond. Unfortunately, the chromatographic separation of the two diastereoisomers was difficult both on the products with the triple bond and on those with the double. In *Figure 4.2* the TLC of the reduction was reported: it is possible to see that the four compounds have similar R_f and their spots are very close on TLC (ETP:AcOEt 4:1), making the separation troublesome.

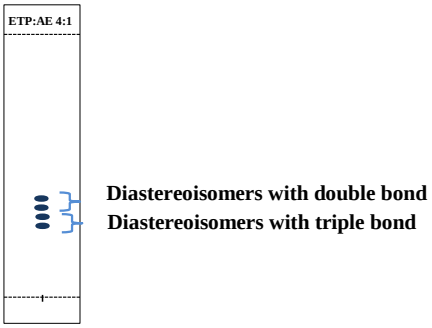
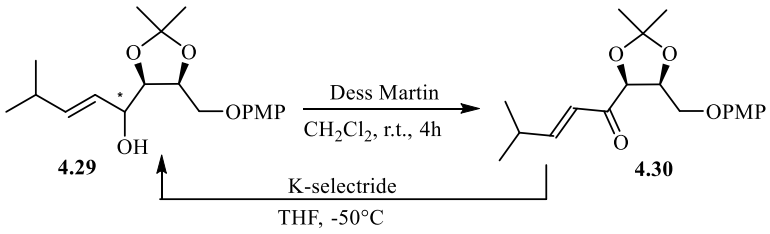


Figure 4.2 TLC of the reduction on both diastereoisomers

Therefore, we decided to perform the reaction after separation, on the major diastereoisomer of the substrate **4.19** *(anti*). With the optimized condition the reaction went to completion, leading to the product **4.29** with 81% yield.

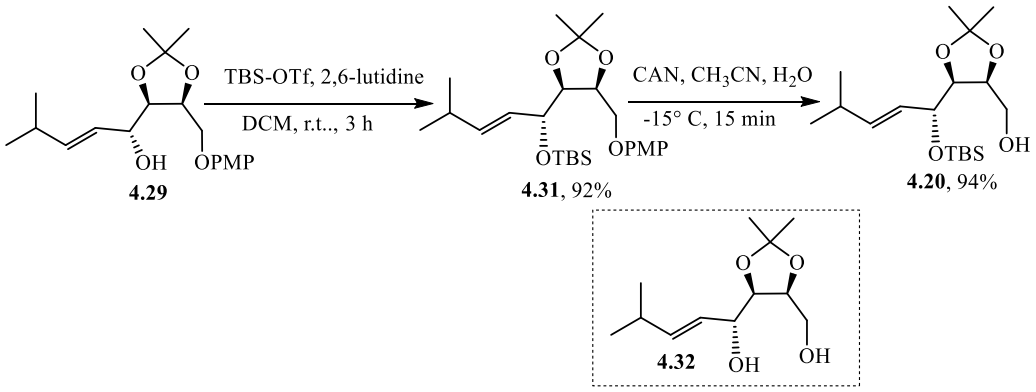
We also tried to carry out the oxidation/reduction sequence on the two diastereoisomers of product **4.29** in order to verify if a higher stereoselectivity could be obtained (*Scheme 4.15*). The same conditions previously optimized were used for the reduction step (K-selectride) but it was less stereoselective than on substrate **4.19** (d.r. 52:48 *syn*: *anti*).



Scheme 4.15 Oxidation to ketone and diastereoselective reduction

Once we obtained compound **4.29**, we planned to protect the primary hydroxy group as TBS and to remove the PMP protecting group in order to later perform Passerini reaction. As previously said, the choice of these two protecting groups was due to their orthogonally behaviour.

The protection reaction with TBS was conducted under the conditions previously optimized in the group (*Scheme 4.16*), affording the product **4.31** with high yield (92%).



Scheme 4.16 Protection with TBS group and cleavage of PMP group

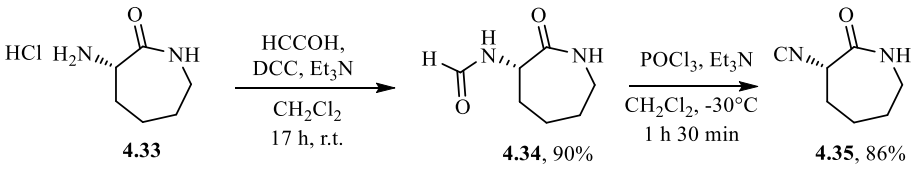
The subsequent cleavage of PMP group was accomplished using the reagent ammonium cerium (IV) nitrate (CAN) in CH_3CN: H_2O 3:1. This step is particularly delicate because the acidic environment of the reaction can also promote the release of the silyl group. In particular, the formation of compound **4.32** has been observed. For this reason, the reaction must take place in a very short time, about 10-15 minutes, and it is extremely important to block it promptly. In the previous work, the excess of oxidant had been removed by $NaHCO_3$ / AcOEt or 10% $Na_2S_2O_5$. However, the formation of several by-products, that could not be removed during the chromatography, had been observed. The problem was solved changing the work up conditions; it was carried out with $NaHCO_3$: 10% $Na_2S_2O_5$ in a 1: 1 mixture. In this way, the excess of oxidant is destroyed and benzoquinone resulted is reduced to hydroquinone that can be easily removed during the extraction, since it is soluble in

water. Thus, the clean product **4.20**, first building blocks for the subsequent Passerini reaction, was obtained with a 94% yield.

4.2.4 Synthesis of isocyanide

In the previous paragraphs the synthesis of the first building block, alcohol **4.20**, necessary for the Passerini reaction has been described. In this paragraph the synthesis of the second building block, isocyanide **4.35**, is reported.

Isocyanide **4.35** was synthesized following a previously optimized procedure, starting from commercially available L-(-)-α-amino-ε-caprolactam **4.33** via two steps sequence (*Scheme 4.17*). The first step involves the formation of the corresponding formamide through the coupling reaction with formic acid, in presence of DCC and Et_3N. The product can be isolated by filtration, avoiding an aqueous work up; this is a great advantage in our case since formamide **4.34** has a high affinity towards water and, therefore, the extraction would be troublesome. Then, the dehydration reaction was carried out to obtain the final product. After an optimization conducted in the previous work, $POCl_3$ was selected as dehydrating agent for the reaction. Even if an extractive work up is necessary to recover the product **4.35**, making the procedure longer due to the poor solubility of the product in organic solvent, the use of $POCl_3$ gave the best results in term of yield.



4.33 **4.34**, 90% **4.35**, 86%

Scheme 4.17 Synthesis of isocyanide

As stated at the beginning of this chapter, particular attention was dedicated to the control of the preservation of optical purity of the products. Thus, the optical purity of isocyanide **4.35** was checked as well. In fact, it is well known that chiral isocyanides bearing an amide or ester group in α position tend to racemize during their preparation. HPLC studies, conducted by the group before my research, demonstrated that the stereochemical information was maintained during the synthetic steps.

4.2.5 Oxidation and diastereoselective Passerini reaction

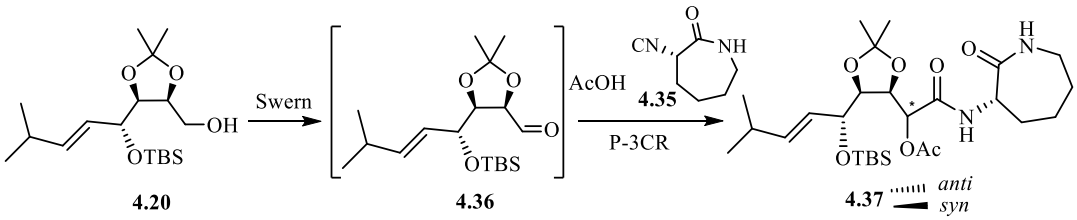
The synthesized building blocks alcohol **4.20** and isocyanide **4.35** were subsequently used with AcOH, to accomplish, after oxidation to aldehyde, the diastereoselective Passerini multicomponent reaction. Based on researches conducted by the group[14], oxidation reaction had been initially performed, in a previous work, on a model compound derived from *meso* diol, under Swern conditions or with one-

pot TEMPO/BAIB procedure. These studies had demonstrated that best results in terms of yield and d.r. could be obtained exploiting Swern condition followed by Passerini reaction. In addition, as also observed in the first project (*Chapter 3*), Swern procedure avoided the overoxidation of the aldehyde to carboxylic acid.

Before describing the results obtained during my work, two other aspects, previously investigated, have to be considered:

a. The maintenance of stereochemical information of the building blocks, isocyanide and alcohol, was verified also during the multicomponent reaction by HPLC analysis. The racemization of the products was not observed, confirming the stability of these compounds in the reaction conditions.
b. The configuration of the newly formed stereogenic centre of the Passerini reaction products was established by analogy with the *meso* diol derived Passerini products obtained in previous works[14a, 14b].

Alcohol **4.20** was oxidized to the corresponding aldehyde **4.36** under Swern condition and the crude product was directly subjected to Passerini reaction (*Scheme 4.18*).



4.20 **4.36** **4.37**

Scheme 4.18 Swern oxidation and Passerini reaction

Swern oxidation was carried out under classical conditions. As for aldehyde **4.24**, we found further drying procedures and the direct use without chromatography to be beneficial. Passerini reaction was initially studied using acetic acid and isocyanide **4.35** as reagents in dry CH_2Cl_2 without any additives. However, following this procedure, we envisioned some problems, described below.

In previous experiments, it had been observed that isocyanide **4.35** is not very reactive (Passerini reaction was slow and difficult to go to completion) and tends to hydrate to the corresponding formamide **4.34** in the reaction environment. To solve these problems, we tried to slowly add the isocyanide, through a syringe pump, to a solution of acetic acid and a careful dried aldehyde **4.36** (*Figure 4.3*).

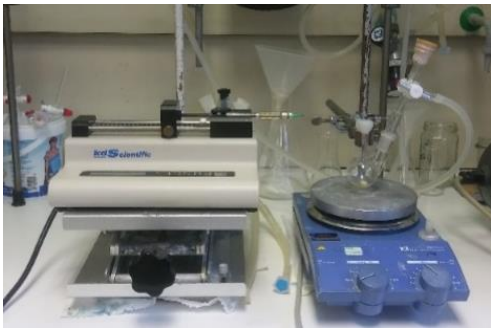
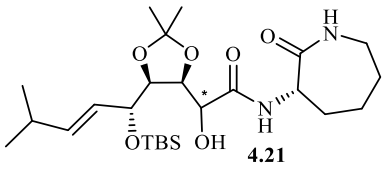


Figure 4.3 Addition of isocyanide through syringe pump

With this method, the presence of the formamide was not observed in the reaction (checked by TLC), indicating that the slow addition was functional. Nevertheless, the product was obtained with a low yield (41% yield in two steps).

The first attempt to perform the diastereoselective Passerini reaction had shown high diastereoselection (87:13 *anti*: *syn*) but a small amount of truncated Passerini products (*Scheme 4.19*) was detected. It doesn't constitute a big problem because the next step consists in a deacetylation reaction. However, by HPLC analysis we observed that the deacetylation mainly occurs on the *syn* diastereosimer of the Passerini products and, therefore, considering only the two acetylated Passerini products a false value of d.r. is obtained. Consequently, the initially d.r. of 87:13 could be lower since *syn* diastereoisomer might have selectively hydrolysed during the reaction.

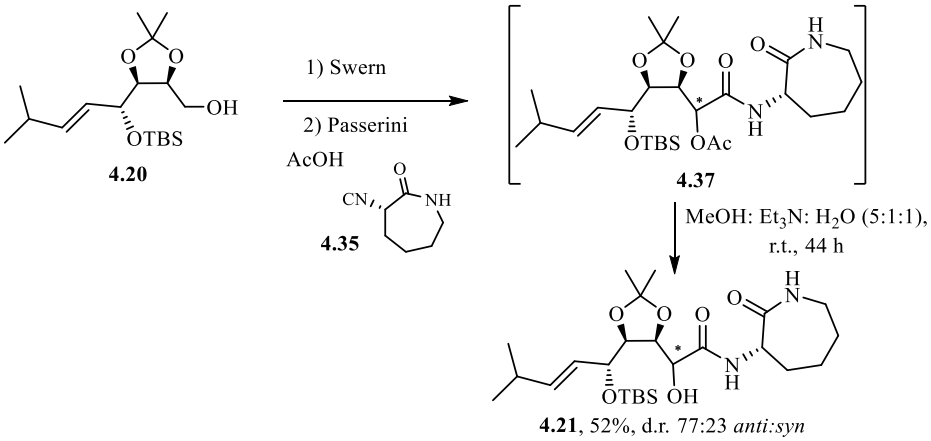


4.21

Scheme 4.19 Truncated Passerini product

Another problem is that the two Passerini products cannot be easily separated by column chromatography. So only a small amount of the desired diastereoisomer (*anti*) can be obtained.

To solve the two last problems, the formation of truncated products and the difficult separation of the two diastereoisomers, we decided to perform Swern oxidation, Passerini reaction and deacetylation in a tandem process in order to avoid the chromatography of the Passerini products and to directly determine the d.r. on the final products **4.21** (*Scheme 4.20*).



Scheme 4.20 Swern oxidation, Passerini reaction and deacetylation reaction

Swern oxidation was performed under classical conditions to obtain the aldehyde which was subsequently used in Passerini reaction with isocyanide (2 eq) and acetic acid (2 eq). CH_2Cl_2 was chosen as solvent and all the reagents were added at the same time (not slowly addition) in the reaction environment. The reaction was stirred for 25 hours at room temperature. Once we obtained the Passerini products **4.37**, the crude product was filtered on silica and then directly subjected to deacetylation reaction with MeOH: Et_3N: H_2O (5:1::1). The reaction was monitored by HPLC analysis (starting material **4.37** and product **4.21** have the same R*f* in TLC) and it afforded the desired product as a mixture of diastereoisomers with 52% yield on three steps and d.r. 77:23 *anti: syn* (*Figure 4.5*).

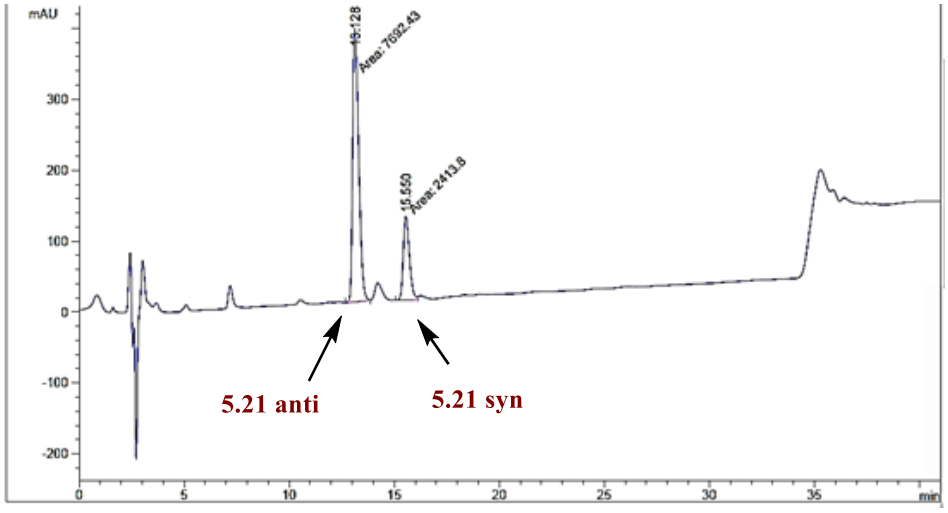


Figure 4.4 HPLC chromatogram of deacetylated products 4.21

Further optimization was conducted on the reaction in order to investigate solvent effects on diastereoselectivity and yield. Based on some results of previous works[14b, 14c], the protocol used was the tandem sequence, using THF or iPr_2O as

solvent. As shown in *Table 4.2*, in both cases the reaction requires more time. However, improved results in terms of yield were obtained using iPr_2O as solvent. Moreover, it was clear that the solvent did not affect the diastereoselection (in all reactions the d.r. was about 8:2 *anti: syn*).

Table 4.2 Solvent optimization for Passerini reaction

Entry	*Reagents*[a]	*Time*	*Temperature*	*Solvent*[b]	*Yield*[c]	*dr*[d] *(anti:syn)*
1	**4.32** AcOH	25 h	r.t.	THF dry	63%	77:23
2[e]	**4.32** AcOH	21 h	r.t.	iPr_2O dry	74%	80:20

[a] Reactions were performed with 2 eq of isocyanide and AcOH, [b] solvents were dried on molecular sieves 4Å, [c] on three steps: Swern oxidation, Passerini reaction and deacetylation, [d] determined by HPLC analysis on the crude product of deacetylation reaction, [e] partial solubility of isocyanide.

To improve the diastereoisomeric ratio we decided to investigate the possible beneficial effect of a Lewis acid additive. Structurally similar chiral aldehydes derived from erythritol have been shown to benefit of the presence of a catalytic amount of $ZnBr_2$[14a] (as diffusely discussed in *Paragraph 3.2.3*). Due to the insolubility of $ZnBr_2$ in CH_2Cl_2, the reaction was only performed in THF and iPr_2O.

Table 4.3 Passerini reaction with $ZnBr_2$

Entry	*Reagents*[a]	*Time*	*Temperature*	*Solvent*[b]	*Yield*[c]	*dr*[d] *(anti: syn)*
1	**4.32** AcOH $ZnBr_2$	20 h	r.t.	THF dry	20%	67:33
2[e]	**4.32** AcOH $ZnBr_2$	20 h	r.t.	iPr_2O dry	14%	80:20

[a] Reactions were performed with 2 eq of isocyanide and AcOH and 0.4 eq of $ZnBr_2$, [b] solvents were dried on molecular sieves 4Å, [c] on three steps: Swern oxidation, Passerini reaction and deacetylation, [d] determined by HPLC analysis on the crude product of deacetylation reaction, [e] partial solubility of isocyanide and $ZnBr_2$.

We can see (*Table 4.3*) that in all cases, the addition of $ZnBr_2$ decreased the yield, as expected, but unlike in the case of other aldehydes deriving from the erythritol it did not lead to a significant improvement on the observed diastereoselectivity.

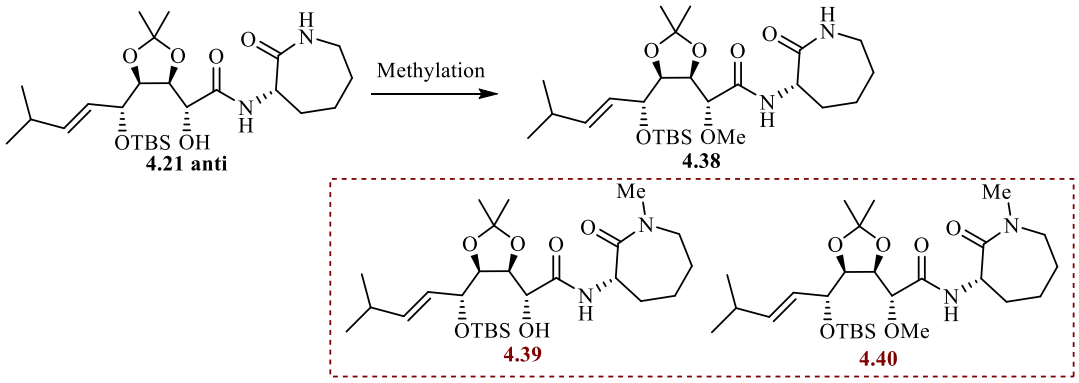
Our final attempt to improve the diastereoselection of the process was to slowly add the components of the Passerini reaction to the reaction mixture. In this way, we

hoped to be able to slow down the reaction rate, thus increasing the possibility of diastereofacial differentiation. We decided to slowly add (in 2 hours) a mixture of aldehyde and acid to the isocyanide and $ZnBr_2$. However, once again the products were obtained with poor yield (19%) and no benefit to the diastereoselection was observed (d.r. 82:18 *anti:syn*).

4.2.6 Synthesis of 4-*epi*-Bengamide E

To obtain 4-*epi*-Bengamide E, methylation reaction and cleavage of the protecting groups (silyl ether and isopropylidene) should be performed on the diastereoisomer *anti* of the product **4.21**.

Concerning methylation reaction, first results had been obtained before my research using MeI and NaH at 0°C. In this condition, product **4.38** had been obtained with other two products, the N-methylated compound **4.39** and the bis-methylated **4.40** (*Scheme 4.21*). In fact, if we consider compounds **4.21**, three sites for the attack of methylating agent are possible: the secondary hydroxy group, the amide group generated during Passerini reaction, and the amide group of the caprolactamic unit.



Scheme 4.21. Methylation reaction

The formation of the side-products was checked by HPLC-MS analysis on the crude product (*Figure 4.6*) and the structures were confirmed by NMR spectra (^1H, ^{13}C, HSQC). By HPLC-MS analysis, it is possible to distinguish the peaks of compound **4.21** (t_r = 13.8 min, m/z = 499.2), **4.39** (t_r = 16.6 min, m/z = 513.2) and **4.40** (t_r = 22.3 min, m/z = 527.2).

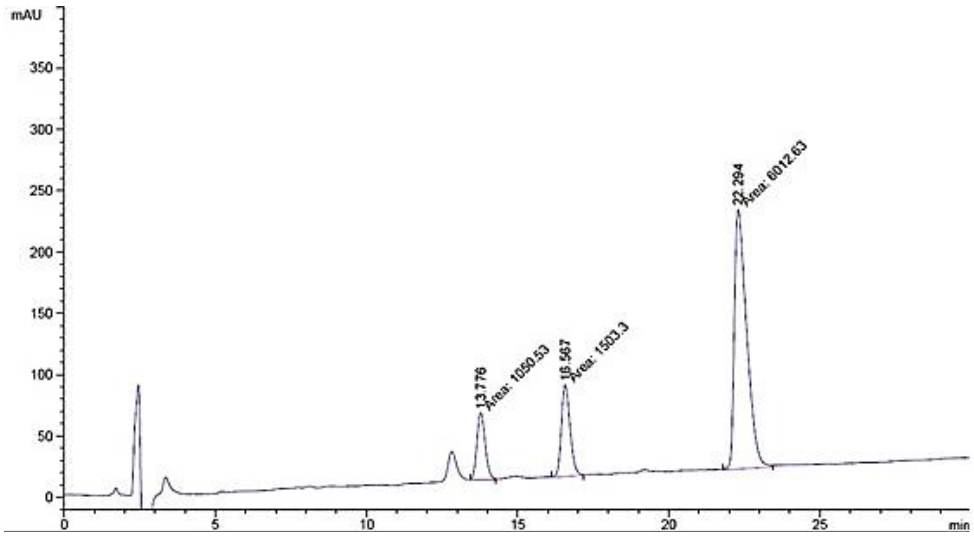


Figure 4.5 Chromatogram of methylation reaction

To improve the formation of the desired product **4.38** and limit the formation of the two side-products, a very slight excess of the base NaH has to be added. In this way, only the desired hydroxy group should be deprotonated lead preferentially to the *O*-methylated product. Nevertheless, NaH is commercially available as 60% suspension in silicon oil that is very difficult to weight, especially in small quantity. Therefore, an addition of a precise quantity of NaH to the reaction mixture is troublesome. To solve this problem, we decided to perform the reaction with different systems.

Firstly, we tried the reaction using the Meerwein's reagent $(CH_3)_3OBF_4$ (1.2 eq) in presence of proton sponge (2 eq), 3Å MS in dry CH_2Cl_2 (0.05 M) at -10°C. Trimethyloxonium tetrafluoroborate is a strong methylating agent; therefore, it can be used with weaker base and, consequently, it could decrease the formation of side-products. Unfortunately, only *N*-methylated derivative **4.39** was obtained (checked by HPLC analysis).

Other two attempts (*Table 4.4*) were performed using MeI and lithium or sodium hexamethyldisilazane. These reactions were carried out on the *syn* diastereoisomer of the product, since we had a small quantity of the *anti*-product. Obviously, once obtained good results, the reaction should have been performed on the *anti* diastereoisomer. The reaction with LiHMDS afforded product **4.39** as well, meanwhile the use of NaHMDS did not gave any product, probably due to the bad quality of the reagent.

Table 4.4 Optimization of methylation reaction on *syn* diatereoisomer

Entry	*Reagents*	*Base*	*Temperature*	*Solvent*	*Products*
1	MeI	LiHMDS[a]	0°C→r.t.	THF	**4.39**
2	MeI	NaHMDS	0°C→r.t.	THF	-

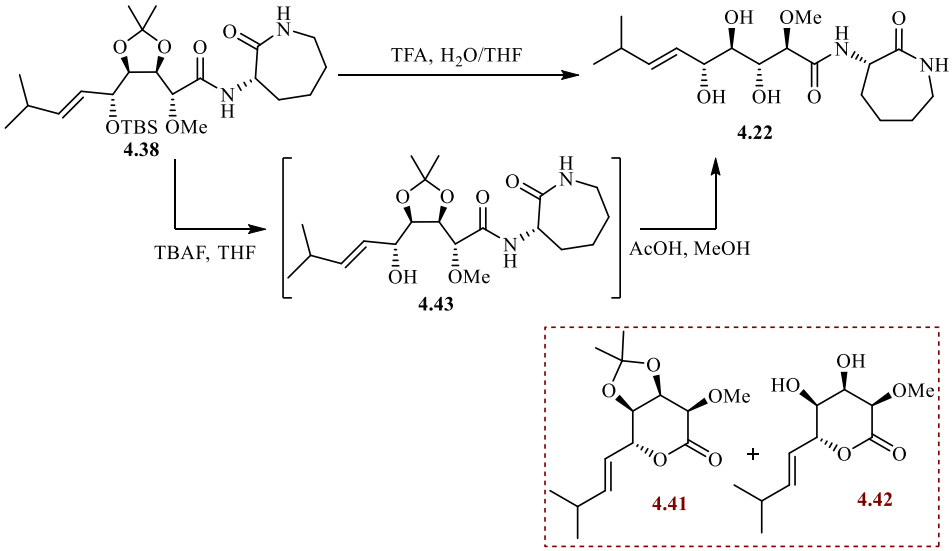
[a] Prepared in situ from HMDS and *n*BuLi

In all the three reactions above the presence of the bis methylated compound was not observed.

From these results, we decided to use the initial conditions (NaH and MeI). To prevent the formation of the bis methylated product, only a small excess of NaH (1.2 eq) was used and the reaction was performed at -10°C instead of 0°C in THF dry (0.2 M). We also tried to add the base in small aliquots (0.6 + 0.6 eq) to the reaction to avoid the presence of an excess of the base in the reaction environment. The reaction is difficult to checked since starting material **4.21** and product **4.38** have the same R*f* in TLC. Unfortunately, the reaction was slow and after 24 h did not go to completion (starting material: product ratio of 45:55). Therefore, we attempted to increase the conversion of the reaction using dry DMF as solvent. Thus, we performed again the reaction on the crude product of the previous one, with 1 eq of NaH in dry DMF. We observed an improvement in the conversion (the reaction was stopped with a starting material: product ratio of 5:95, determined by HPLC analysis on the crude product), but at the same time a major quantity of bis methylated product **4.40** and the formation of *N*-methylated product **4.39**. The increased formation of the side-products (20% yield of **4.40** and 3% of **4.39**) can be explained by the use of an excess of NaH respect to the starting material, that was added to lead the reaction to completion. In addition, probably DMF solvent makes the methylating agent too reactive and consequently less selectivity for *O*-methylation. Other experiments should be performed in THF to limit the formation of side-products, monitoring the reaction with HPLC analysis to be sure to stop the reaction after completion.

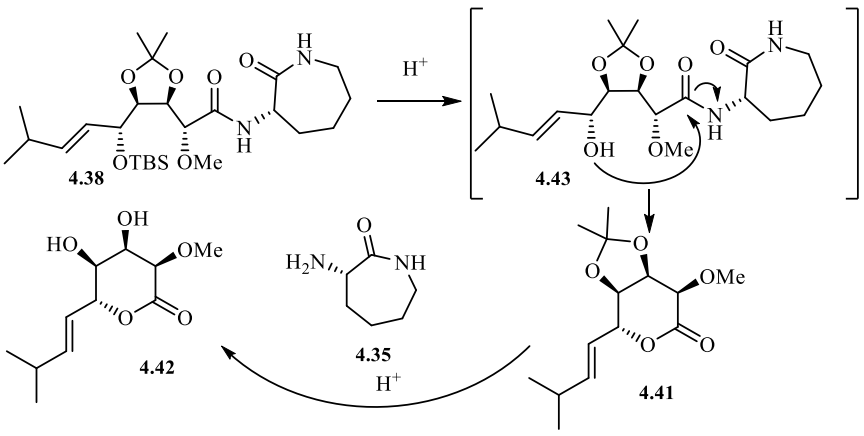
The last step of the synthesis involves the cleavage of the protecting groups (silyl ether and isopropylidene groups). Both these functionalities can be removed under acidic conditions, therefore, theoretically the final cleavage could be performed in only one step.

Some preliminary studies had been done before my research. In particular, two different conditions had been attempted for the final cleavage (*Scheme 4.22*).



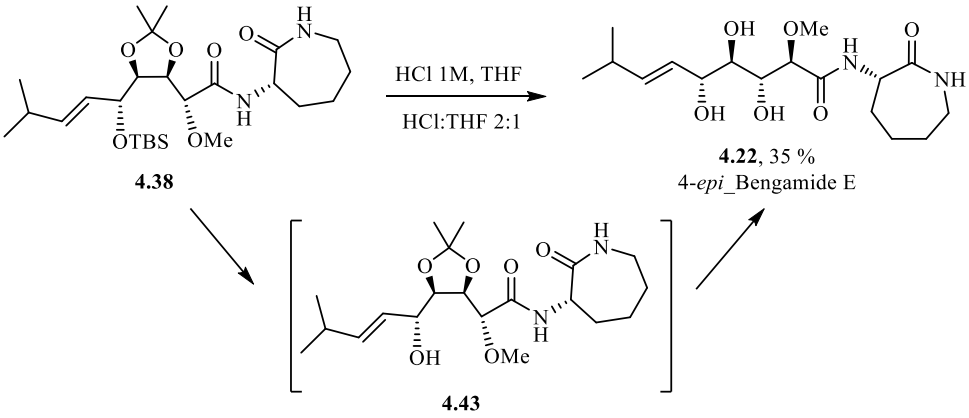
Scheme 4.22 Previous results of final cleavage

The first one involved a one-step procedure with TFA in H_2O/THF. In the second attempt a two steps sequence, as reported by Sarabia[15], was performed. It involves the cleavage of TBS group with TBAF in THF and the subsequent reaction with AcOH 70% (aqueous solution) in MeOH to remove the acetonide group. In both cases the product **4.22** was obtained with low yields and two other side-products **4.41** and **4.42** were observed (*Scheme 4.22*). The formation of these products is probably due to the intramolecular attack of OH to the carbonyl group of the amide. The possible mechanism involves the initial cleavage of *t*-butyldimethylsilyl ether which is easily hydrolysed in the acidic environment. The spatial proximity between the hydroxy group and the amide group favours the intramolecular attack of OH to the carbonyl group of the amide to form the corresponding lactone **4.41** and expelling the amino-caprolactam **4.33**. Once the lactone is formed, the acidic environment promotes the release of acetonide, giving rise to lactone **4.42**, containing two free hydroxy groups (*Scheme 4.23*).



Scheme 4.23 Possible mechanism for the formation of lactones

Taking into account the previous results, we decided to perform a different strategy for the cleavage of the protecting groups using HCl 1M in THF (*Scheme 4.24*)[16]. In these conditions, the product **4.22** was obtained with a higher yield (35%) and only a small quantity of cyclized products was obtained. We also observed the presence of the product without the TBS group but still with the acetonide group. However, it can be easily eliminated by stirring the reaction for a longer time.



Scheme 4.24 Cleavage with HCl 1 M

4.3 Conclusion

In summary, the total synthesis of 4-*epi*-Bengamide E, a stereoisomer of Bengamide E never synthesized before, was successfully completed applying a new synthetic route.

This new strategy demonstrates the efficiency of coupling biocatalysis and MCRs starting from bio-based products to access a densely functionalized and stereochemically complex compound. The four contiguous stereocentres are generated with high stereocontrol: the enzymatic desymmetrization delivers 95% e.e. (in favour of the desired enantiomer), whereas the diastereoselective reduction of ketone and the Passerini reaction provide the required diastereoisomer with good selectivity. In addition, using the same approach different analogues of Bengamides could be synthesized by variation of the reagents.

Studies on the optimization of the final steps (methylation and final cleavage) are in progress. The last results obtained showed an increase in the yields for both the reactions. Performing the methylation with NaH (1.5 eq) and MeI (2 eq) in THF dry for 2 days at -10°C the reaction went to completion (monitored by HPLC analysis) and the product was obtained with 63% yield. Concerning the final cleavage, it was carried on with a higher concentration of HCl (2 M) affording the final product with 43% yield.

The extension of this synthetic strategy to the natural Bengamide E will require some modifications in order to selective epimerized the 4-OH. Some further investigation in this direction will be performed.

4.4 Experimental Section

4.4.1 General remarks

NMR spectra were taken at room temperature in $CDCl_3$ or in d_6-DMSO at 300 MHz (^1H), and 75 MHz (^{13}C), using, as internal standard, TMS (^1H-NMR: 0.000 ppm) or the central peak of $CDCl_3$ (^{13}C: 77.160 ppm). Chemical shifts are reported in ppm (δ scale). Coupling constants are reported in Hertz. Resonances are described as s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet) and m (multiplet) or combinations thereof. Peak assignments were made with the aid of gCOSY and gHSQC experiments. In ABX system, the proton A is considered upfield and B downfield.

GC-MS were carried out using an HP-1 column (12 m long, 0.2 mm wide), electron impact at 70 eV, and a mass temperature of about 170 °C. Only m/z > 33 were detected. All analyses were performed (unless otherwise stated) with a constant He flow of 1.0 ml/min with initial temp. of 70 °C, init. time 2 min, rate 20 °C/min, final temp. 260 °C, inj. temp. 250 °C, det. temp. 280 °C.

HPLC analyses for the determination of enantiomeric ratios were performed on a Daicel Chiral Pak AD 250x4.6 mm column, at 25–28 °C with a flow of 1 mL/min (UV detection at λ = 220 nm).

HPLC-MS analyses were performed on Synergi Hydro RP 150x3 mm column, at 30 °C with a flow of 0.5 mL/min (where not otherwise stated). HRMS: samples, provided at 10mM in DMSO, were analysed on a UPLC Acquity system coupled to a Synapt G2 QToF mass spectrometer. MS signals were acquired from 50 to 1200 m/z both ESI positive and ESI negative ionization mode.

IR spectra were recorded directly on solid, oil, or foamy samples with the ATR (attenuated total reflectance) technique, using a FT Perkin Elmer Spectrum 65 spectrophotometer.

Optical rotations were measured with a JASCO P-2000 digital polarimeter, using the sodium D line as the light source. The specific rotation $[\alpha]^D$ was calculated as: $[\alpha]_D = \alpha * 100 / c * l$ (α = observed rotation of solution; l = length of polarimeter tube in decimetres, c = concentration of the solution in g/100 mL in $CHCl_3$).

Melting points were determined with an electrothermal apparatus (Büchi B-535).

TLC analyses were carried out on silica gel plates and viewed at UV (254 nm) and developed with Hanessian stain (dipping into a solution of $(NH_4)_4MoO_4 \cdot 4H_2O$ (21 g) and $Ce(SO_4)_2 \cdot 4H_2O$ (1 g) in H_2SO_4 (31 mL) and H_2O (469 mL) and warming).

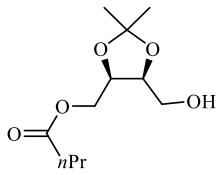
R_f values were measured after an elution of 7-9 cm.

Column chromatographies were done with the "flash" methodology using 220-400 mesh silica.

All reactions using dry solvents were carried out under a nitrogen or argon atmosphere.

4.4.2 Experimental procedures

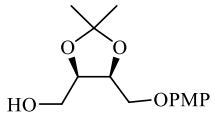
4.4.2.1 Synthesis of chiral building blocks



((4R,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl butyrate 4.18

Diol **4.15** (3.592 g, 22.15 mmol) was dissolved in THF dry (55.4 mL) under nitrogen atmosphere. Molecular sieves 3Å (1.107 g, 50mg/mol), vinyl butyrate (14 mL, 110.74 mmol) and Amano PS lipase (1.796 g) were added and the reaction was stirred at 20 °C for 32 h. Then it was filtered on celite washing with DCM and concentrated. The residue was purified by column chromatography on silica gel (ETP/AcOEt 6:4), to give alcohol **4.18** as a colourless oil (4.629 g, 96% yield, conversion: 56%). The analytical data conform to those reported in literature[17].

4.15, colourless oil, R*f* = 0.0.38 (ETP:AcOEt 3:2), developed with Hanessian stain.
$[\alpha]^{20}_{D}$= + 13.70 (c = 1.04, $CHCl_3$)
HRMS (ESI+): m/z calcd for $C_{11}H_{20}O_5$ $[M+Na]^+$: 255.1208; found: 255.1198



((4R,5S)-5-((4-methoxyphenoxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol 4.23

PMP protection

A solution of alcohol **4.18** (4.629 g, 21.21 mmol) in dry CH_2Cl_2 (212 mL) was treated with triphenylphosphine (8.345 g, 31.81 mmol) and *p*-methoxyphenol (7.899 g, 63.63 mmol), under nitrogen atmosphere. Then *tert*-butyl azodicarboxylate (7.326 g, 31.81 mmol) was added slowly at 0°C. The reaction mixture was then stirred for 26 h at room temperature. The solvent was removed by evaporation under reduced pressure and the residue was filtered on silica gel (ETP: DCM: Et_2O 1:1:1) and directly used for the next reaction.
*R*f = 0.9 (ETP:AcOEt 6:4), developed with Hanessian stain.

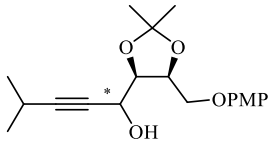
Butyrate cleavage

To a solution of crude compound (21.55 mmol) in MeOH (76 mL), KOH (1M, 1.815 g, 32.35 mmol solubilized in 32 mL of MeOH) was added. The reaction was stirred at room temperature for 6 h and then it was quenched with 100 mL of NH_4Cl (sat) and extracted with Et_2O. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. In order to remove the precipitate (*p*-methoxyphenol and hydrazine carboxylate), the residue was diluted and filtered on celite and purified by flash column chromatography on silica gel (ETP: AcOEt 2:1 and ETP: AcOEt 1:1) to give 4.16 g (89% yield in 2 steps) of a pale yellow solid. e.e. 95%

4.23, pale yellow solid, R*f* = 0.27 (ETP:AcOEt 2:1), developed with Hanessian stain.
$[\alpha]^{24}_{D}$= + 8.19 (c = 1.04, $CHCl_3$), m.p. = 52.3 °C- 54.2 °C

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.95 – 6.77 (m, 4H, CH Ar.), 4.54 (q, J = 6.3 Hz, 1H, C<u>H</u>CH_2OPMP), 4.39 (q, J = 6.0 Hz, 1H, C<u>H</u>CH_2OH), 4.04 (d, J = 6.2 Hz, 2H. C<u>H_2</u>OPMP), 3.88 – 3.73 (m, 2H, C<u>H_2</u>OH), 3.77 (s, 3H, OC<u>H_3</u>), 2.28 (s, 1H, OH), 1.50 (s, 3H, CH_3 of *i*Pr), 1.41 (s, 3H, CH_3 of *i*Pr). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 154.48 (C quat. Ar), 152.33 (C quat. Ar), 115.72 (2 CH Ar), 114.84 (2 CH Ar), 109.02 (<u>C</u>$(CH_3)_2$), 77.39 (<u>C</u>HCH_2OH), 75.05 (<u>C</u>HCH_2OPMP) 67.29 (<u>C</u>H_2OPMP), 61.14 (<u>C</u>H_2OH), 55.86 (O<u>C</u>H_3), 27.88 (CH_3 of *i*Pr), 25.29 (CH_3 of *i*Pr). *IR* ν (cm^{-1}) = 3519; 3058; 2988; 2938; 2887; 2836; 1509; 1460; 1374; 1335; 1289; 1216; 1182; 1164; 1111; 1089; 1050; 1035; 996; 907; 845; 830; 818; 803; 751; 715; 650. *HRMS* (ESI+): m/z calcd for $C_{14}H_{20}O_5$ $[M+Na]^+$: 291.1209; found: 291.1106.

4.4.2.2 Synthesis of propargylic alcohol



1-((4R,5S)-5-((4-methoxyphenoxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-4-methylpent-2-yn-1-ol 4.19

<u>Swern oxidation</u>

To a solution of DMSO (1.1 mL, 15.08 mmol) in dry CH_2Cl_2 (36 ml), at –78 °C under nitrogen atmosphere, a solution of oxalyl chloride in dry CH_2Cl_2 (1.43 M, 9.8 mL) was added. Upon completion of the addition, the mixture was stirred at -78 °C for 10 min. A solution of alcohol **4.23** (1.499 g, 5.59 mmol) in dry CH_2Cl_2 (20 ml) was added dropwise, and the solution was stirred for 10 min at –78 °C. Then Et_3N (4.3 mL, 30.73 mmol) was added and the solution was stirred for 2 h (the reaction was monitored by TLC and ^{1}H-NMR). The reaction was quenched by addition of 5% aq. $(NH_4)H_2PO_4$ (130 mL + 1 N HCl solution) to have a final pH = 4 and extracted with Et_2O (3 x 60 mL). The combined organic layers were washed with brine (60 mL) and dried (Na_2SO_4, dried in an oven at 100°C for one night and cooled under vacuum). In order to remove water, the organic phase was filtered on celite and Na_2SO_4 and concentrated to afford aldehyde **4.24** as a yellow oil, which was immediately used for the next reaction without any further purification.

R*f* = 0.54 (ETP:AcOEt 6:4), developed with Hanessian stain.

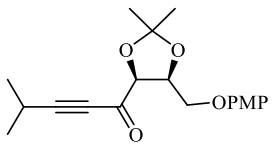
<u>Addition of acetylide</u>

In a 3-necks flask 2,2-bipyridyl and dry THF (30 mL, dried over molecular sieves 4 A°) were added under argon atmosphere. Then the mixture was cooled to -50°C and *n*BuLi (11 mL, 1.6 M in hexane) and 3-methyl-1-butyne (2 mL, 19.55 mmol) were added. The reaction was stirred for 30 min (until the red colour disappeared) and a solution of crude aldehyde (5.59 mmol) in 20 mL dry THF was added dropwise (15 min). The reaction mixture was stirred 1 h at -50°C and then it was allowed to reach room temperature and stirred overnight. The reaction was quenched with 100 mL of NH_4Cl (sat) and extracted with AcOEt (3 x 60 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography on silica gel (ETP: Et_2O 3:1) to give **4.19** (1.587 g, 88% yield in two

steps) as a mixture of diastereoisomers (d.r. = 57: 43 *anti*: *syn*, calculated by HPLC analysis on the crude product).

4.19_*anti* pale yellow foam; R*f* = 0.73 (ETP:AcOEt 6:4), developed with Hanessian stain. $[\alpha]^{24}_D$= -13.01 (c = 1.00, $CHCl_3$)
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 6.93 –6.79 (m, 4H, CH Ar.), 4.63 – 4.56 (m, 2H, C<u>H</u>CH_2 e C<u>H</u>OH), 4.36 (dd, *J*= 10.1, 5.1 Hz, 1H, C<u>H</u>$_2$OPMP), 4.31 (dd, *J*= 6.5, 5.0 Hz, 1H, C<u>H</u>CHOH), 4.20 (dd, *J*= 10.1, 6.3 Hz, 1H, C<u>H</u>$_2$OPMP), 3.76 (s, 3H, OC<u>H</u>$_3$), 2.79 (d, *J*= 5.7 Hz, 1H, OH), 2.56 (heptd, *J*= 6.9, 1.8 Hz, 1H, C<u>H</u>$(CH_3)_2$), 1.55 (s, 3H, CH_3 of *i*Pr), 1.42 (s, 3H, CH_3 of *i*Pr), 1.13 (d, *J*= 6.9 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 154.07 (C quat. Ar), 152.40 (C quat. Ar), 115.58 (2 CH Ar), 114.53 (2 CH Ar), 109.08 (<u>C</u>$(CH_3)_2$), 92.55 (<u>C</u>≡CCHOH and C≡<u>C</u>CHOH), 79.29 (<u>C</u>HCHOH), 75.45 (<u>C</u>HCH_2OPMP) 67.32 (<u>C</u>H_2OPMP), 61.65 (<u>C</u>HOH), 55.57 (O<u>C</u>H_3), 27.29 (CH_3 of *i*Pr), 25.15 (CH_3 of *i*Pr), 22.62 (2 CH_3 of $CH(CH_3)_2$), 20.42 (<u>C</u>H$(CH_3)_2$). *HRMS* (ESI+): m/z calcd for $C_{19}H_{26}O_5$ $[M+Na]^+$: 357.1678; found: 357.1670.

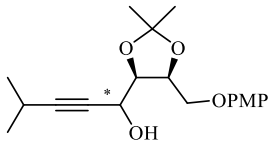
4.19_*syn* pale yellow oil; R*f* = 0.65 (ETP:AcOEt 6:4), developed with Hanessian stain $[\alpha]^{20}_D$= -76.29 (c = 1.03, $CHCl_3$)
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.94 – 6.74 (m, 4H, CH Ar.), 4.63 – 4.47 (m, 2H, C<u>H</u>CH_2OPMP and C<u>H</u>OH), 4.39 – 4.23 (m, 2H, C<u>H</u>CHOH and C<u>H</u>$_2$OPMP), 4.18 – 3.92 (m, 1H, C<u>H</u>$_2$OPMP), 3.77 (s, 3H, OC<u>H</u>$_3$), 2.76 – 2.31 (m, 1H, C<u>H</u>$(CH_3)_2$), 1.54 (s, 3H, CH_3 of *i*Pr), 1.43 (s, 3H, CH_3 of *i*Pr), 1.13 (dd, J = 6.9, 2.0 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 154.21 (C quat. Ar), 152.70 C (quat. Ar), 115.65 (CH Ar.), 114.68 (CH Ar.), 109.62 (<u>C</u>$(CH_3)_2$), 93.05 (<u>C</u>≡CCHOH and C≡<u>C</u>CHOH), 79.84 (<u>C</u>HCHOH), 75.57 (<u>C</u>HCH_2OPMP), 67.18 (<u>C</u>H_2OPMP), 61.32 (<u>C</u>HOH), 55.83 (O<u>C</u>H_3), 27.80 (CH_3 of *i*Pr), 25.46 (CH_3 of *i*Pr), 22.81 (2 CH_3 of $CH(CH_3)_2$), 20.59 (<u>C</u>H$(CH_3)_2$). *IR* ν (cm^{-1}) = 3455; 3222; 2970; 2934; 2835; 1507; 1458; 1381; 1319; 1289; 1228; 1214; 1167; 1125; 1106; 1082; 1038; 884; 856; 824; 727; 639. *HRMS* (ESI+): m/z calcd for $C_{19}H_{26}O_5$ $[M+Na]^+$: 357.1678; found: 357.1670.



1-((4S,5S)-5-((4-methoxyphenoxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-4-methylpent-2-yn-1-one 4.28
Compound **4.19** (both diastereoisomers) (1.476 mg, 4.41 mmol) was dissolved in 44 mL of dry CH_2Cl_2 under nitrogen atmosphere. Then Dess Martin periodinane (2.00 g, 4.85 mmol) was added to the solution at 0°C and the reaction was stirred at room temperature for 3 h and 30 minutes. The reaction was quenched with 140 mL of a solution 1:1 $NaHCO_3$: $Na_2S_2O_3$ and extracted with CH_2Cl_2 (3 x 60 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography on silica gel (ETP: AcOEt 5:1) to afford **4.28** as a pale yellow foam (1.036 g, 71% yield).

4.28, pale yellow foam, R*f* = 0.50 (ETP:AcOEt 4:1), developed with Hanessian stain.

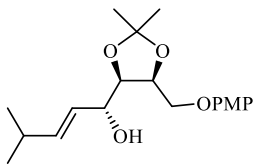
$[\alpha]^{23}_D$= + 16.59 (c = 1.07, $CHCl_3$)
^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.90 – 6.73 (m, 4H, CH Ar.), 4.76 (ddd, J = 7.6, 5.8, 4.0 Hz, 1H, C<u>H</u>CH_2OPMP), 4.67 (d, J = 7.6 Hz, 1H, C<u>H</u>C=O), 4.12 (dd, J = 10.2, 4.0 Hz, 1H, C<u>H</u>$_2$OPMP), 4.00 (d, J = 5.8 Hz, 1H, C<u>H</u>$_2$OPMP), 3.75 (s, 3H, OC<u>H</u>$_3$), 2.71 (hept, J = 6.9 Hz, 1H, C<u>H</u>$(CH_3)_2$), 1.67 (s, 3H, CH_3 of iPr), 1.44 (s, 3H, CH_3 of iPr), 1.20 (dd, J = 6.9, 1.7 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 185.51 (C quat. C=O), 154.27 (C quat. Ar), 152.36 (C quat. Ar), 115.59 (2 CH. Ar), 114.58 (2 C quat. Ar), 111.43 (CHC≡<u>C</u>), 103.64 (<u>C</u>$(CH_3)_2$), 81.76 (<u>C</u>HCH_2OPMP), 79.29 (CH<u>C</u>≡C), 76.91 (<u>C</u>HC=O), 66.47 (<u>C</u>H_2OPMP), 55.74 (O<u>C</u>H_3), 27.04 (CH_3 of iPr), 25.39 (CH_3 of iPr), 21.77 (2 CH_3 of $CH(CH_3)_2$), 20.99 (<u>C</u>H$(CH_3)_2$). *IR* ν (cm^{-1}) = 2976; 2935; 2835; 2205; 1667; 1593; 1507; 1456; 1381; 1317; 1289; 1212; 1163; 1093; 1066; 1036; 985; 909; 890; 857; 823; 798; 743; 709; 663; 636. *HRMS* (ESI+): m/z calcd for $C_{19}H_{24}O_5$ $[M+Na]^+$: 355.1522; found: 355.1504.



1-((4R,5S)-5-((4-methoxyphenoxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-4-methylpent-2-yn-1-ol 4.19

A solution of **4.28** (1.036 g, 3.12 mmol) in dry THF (31 mL) was placed at -78°C under nitrogen atmosphere and K-Selectride (1 M in THF, 31 mL) was added. The reaction was stirred at room temperature for 5 h and then it was quenched with 40 mL NH_4Cl (sat) and extracted with AcOEt (3 x 40 mL). The organic phase was washed with brine (40 mL), dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography on silica gel (ETP: AcOEt 3:1) to afford **4.19** (844 mg, 81% yield) as a mixture of diastereoisomers (d.r. = 74:26 anti: syn, calculated by HPLC analysis on the crude product).

4.4.2.3 Manipulation of functional groups

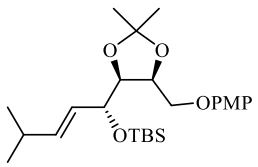


(R, E)-1-((4R,5S)-5-((4-methoxyphenoxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-4-methylpent-2-en-1-ol 4.29

Compound **4.19** (556 mg, 1.66 mmol) was dissolved in 17 mL of dry THF under argon atmosphere. Then Red-Al (3.5 M in toluene, 1.2 mL) was added dropwise to the solution at 0°C and the reaction was stirred under reflux for 4 h. Then it was cooled to 0 °C and carefully quenched with 50 mL of 30% Rochelle salt solution and NH_4Cl (sat.) 1:1. The mixture was stirred for 1 h and then extracted with AcOEt. The organic phase was then washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography on silica gel (ETP: Et_2O 3:1) to afford **4.29** (451 mg, 81% yield) as a white solid.

4.29, white solid, R*f* = 0.55 (ETP:AcOEt 4:1), developed with Hanessian stain.
$[\alpha]^{24}_D$= + 4.52 (c = 1.05, $CHCl_3$). m.p. = 44.6 °C - 47.1°C (CH_2Cl_2)

^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 6.92 – 6.80 (m, 4H, CH Ar.), 5.79 (ddd, *J*= 15.6, 6.5, 1.2 Hz, 1H, C<u>H</u>=CHCHOH), 5.57 (ddd, *J*= 15.6, 5.7, 1.2 Hz, 1H, CH=C<u>H</u>CHOH), 4.62 – 4.47 (m, 1H, C<u>H</u>CH_2), 4.33 – 4.25 (m, 1H, C<u>H</u>OH), 4.19 (dd, *J*= 9.7, 6.8 Hz, 1H, C<u>H</u>$_2$OPMP), 4.13 (dd, *J*= 7.8, 5.7 Hz, 1H, C<u>H</u>CHOH), 4.0 (dd, *J*= 9.7, 5.5 Hz, 1H, C<u>H</u>$_2$OPMP), 3.77 (s, 3H, OCH_3), 2.72 (d, *J*= 3.6 Hz, 1H, OH), 2.45 – 2.22 (m, 1H, C<u>H</u>$(CH_3)_2$), 1.47 (s, 3H, CH_3 of *i*Pr), 1.39 (s, 3H, CH_3 of *i*Pr), 1.02 (d, *J*= 6.8 Hz, 3H, CH_3 of $CH(CH_3)_2$), 1.01 (d, *J*= 6.8 Hz, 3H, CH_3 of $CH(CH_3)_2$). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 154.54 (C quat. Ar), 152.22 (C quat. Ar), 140.59 (<u>C</u>H=CHCHOH), 125.90 (CH=<u>C</u>HCHOH), 115.79 (2 CH Ar), 114.84 (2 CH Ar), 109.02 (<u>C</u>$(CH_3)_2$), 80.24 (<u>C</u>HCHOH), 75.71 (<u>C</u>HCH_2), 70.38 (<u>C</u>HOH) 67.78 (<u>C</u>H_2OPMP), 55.87 (O<u>C</u>H_3), 30.98 (<u>C</u>H$(CH_3)_2$), 28.09 (CH_3 of *i*Pr), 25.55 (CH_3 of *i*Pr), 22.41 (CH_3 of $CH(CH_3)_2$), 22.30 (CH_3 of $CH(CH_3)_2$). *IR* ν (cm^{-1}) = 3487; 2990; 2957; 2939; 2883; 2867; 2837; 1858; 1670; 1624; 1591; 1506; 1458; 1441; 1412; 1379; 1367; 1329; 1302; 1290; 1250; 1220; 1183; 1167; 1137; 1113; 1081; 1039; 1013; 971; 958; 936; 923; 906; 861; 822; 799; 778; 721; 669; 659; 642; 605. *HRMS* (ESI+): m/z calcd for $C_{19}H_{28}O_5$ $[M+Na]^+$: 358.1834; found: 359.1816.

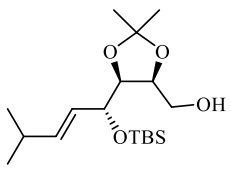


Tert-butyl(((R,E)-1-((4S,5S)-5-((4-methoxyphenoxy) methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-4-methylpent-2-en-1-yl)oxy)dimethylsilane 4.31

Compound **4.29** (381 mg, 1.13 mmol) was dissolved in 5.7 mL of dry CH_2CH_2 under nitrogen atmosphere and 2,6-lutidine (527 μL, 4.53 mmol) was added. Then TBS-OTf (624 μL, 2.72 mmol) was added dropwise to the solution at 0°C and the reaction was stirred at room temperature for 3 h. The reaction was quenched with 10 mL of NH_4Cl (sat.) and extracted with CH_2CH_2 (3 x 10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography on silica gel (ETP: Et_2O 8:1) to give **4.31** (470 mg, 92%) as a colourless oil.

4.31, colourless oil, R*f* = 0.81 (ETP:AcOEt 4:1), developed with Hanessian stain. $[\alpha]^{25}_D$ = -26.25 (c=1.03, $CHCl_3$).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C,TMS): δ = 6.89 – 6.78 (m, 4H, CH Ar), 5.66 (ddd, J = 15.5, 6.6, 1.1 Hz, 1H, C<u>H</u>=CHCHOH), 5.38 (ddd, J = 15.5, 7.0, 1.3 Hz, 1H, CH=C<u>H</u>CHOH), 4.48 (ddd, J = 8.8, 6.4, 2.8 Hz, 1H, C<u>H</u>CH_2), 4.39 (ddd, J = 6.6, 5.5, 1.0 Hz, 1H, C<u>H</u>OTBS), 4.22 (dd, J = 10.2, 2.8 Hz, 1H, C<u>H</u>$_2$OPMP), 4.11 (dd, J = 6.3, 5.4 Hz, 1H, C<u>H</u>CHOH), 4.06 (dd, J = 10.2, 1.8 Hz, 1H, C<u>H</u>$_2$OPMP), 3.77 (s, 3H, OCH_3), 2.38 – 2.23 (m, 1H, C<u>H</u>$(CH_3)_2$), 1.47 (s, 3H, CH_3 of *i*Pr), 1.38 (s, 3H, CH_3 of *i*Pr), 0.99 (d, J = 6.7 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$), 0.90 (s, 9H, CH_3 of *t*Bu), 0.09 (s, 3H, CH_3 of TBS), 0.05 (s, 3H, CH_3 of TBS). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 153.8 (C quat. Ar), 153.2 (C quat. Ar), 140.9 (<u>C</u>H=CHCHOH), 126.5 (CH=<u>C</u>HCHOH), 115.6 (2 CH Ar), 114.5 (2 CH Ar), 108.5 (<u>C</u>$(CH_3)_2$), 79.7 (<u>C</u>HCHOTBS), 76.4 (<u>C</u>HCH_2), 72.7 (<u>C</u>HOTBS), 69.0 (<u>C</u>H_2OPMP), 55.7 (<u>C</u>H_3O),

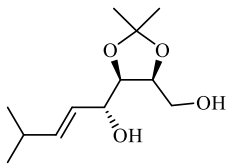
30.8 (CH(CH$_3$)$_2$), 27.9 (CH$_3$ of *i*Pr), 26.0 (3 CH$_3$ of *t*But), 25.5 (CH$_3$ of *i*Pr), 22.1 (CH$_3$ of CH(CH$_3$)$_2$), 22.0 (CH$_3$ of CH(CH$_3$)$_2$), 18.2 (C quat of *t*Bu), -3.6 (CH$_3$ of TBS), -4.4 (CH$_3$ of TBS). *IR* ν (cm^{-1}) = 2956; 2931; 2858; 1669; 1592; 1508; 1463; 1375; 1288; 1222; 1179; 1141; 1080; 1043; 1005; 976; 939; 880; 824; 775; 736; 670. *HRMS* (ESI+): m/z calcd for $C_{25}H_{42}O_5Si$ [M+Na]$^+$: 473.2700; found: 473.2731.



((4S,5S)-5-((R,E)-1-((tert-butyldimethylsilyl)oxy)-4-methyl pent-2-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol 4.20

Compound **4.31** (75 mg, 0.17 mmol) was dissolved in CH$_3$CN (3 mL) and a solution of CAN (1 mL, 0.4M in deionized water) was added dropwise at -15°C. The reaction was stirred for 15 minutes at -15° C. The reaction was quenched with 20 mL of a solution 1:1 NaHCO$_3$: Na$_2$S$_2$O$_3$ and extracted with CH$_2$CH$_2$ (3 x 15 mL). The combined organic layers were dried (Na$_2$SO$_4$) and concentrated. The residue was purified by flash column chromatography on silica gel (ETP: DCM: Et$_2$O 4:1:0.5) to afford **4.20** as a pale yellow oil (54 mg, 94% yield).

4.20, pale yellow oil, R*f* = 0.18 (ETP: DCM: Et$_2$O 4:1:0.5), developed with Hanessian stain. [α]$^{24}_D$= -17.83 (c = 1.24, CH$_3$Cl).
^{1}H NMR (300 MHz, CDCl$_3$, 25 °C,TMS): δ = 5.65 (dd, J = 15.4, 6.6 Hz, 1H, CH=CHCHOTBS), 5.38 (dd, J = 15.5, 7.2 Hz, 1H, CH=CHCHOTBS), 4.43 (t, *J*= 6.3 Hz, 1H, CHOTBS), 4.18 (q, J = 5.7 Hz, 1H, CHCH$_2$), 4.01 (t, J = 5.6 Hz, 1H, CHCHOTBS), 3.91 – 3.71 (m, 2H, CH$_2$OH), 2.95 (t, *J*= 6.9 Hz, 1H, OH), 2.31 (heptd, *J*= 6.6, 1.1 Hz, 1H, CH(CH$_3$)$_2$), 1.44 (s, 3H, CH$_3$ of *i*Pr), 1.34 (s, 3H, CH$_3$ of *i*Pr), 0.99 (dd, J = 6.7, 1.6 Hz, 6H, 2 CH$_3$ of CH(CH$_3$)$_2$), 0.89 (s, 9H, 3 CH$_3$ of *t*Bu), 0.11 (s, 1H, CH$_3$ of TBS), 0.07 (s, 1H, CH$_3$ of TBS). *^{13}C NMR* (75 MHz, CDCl$_3$, 25 °C, TMS): δ = 141.43 (CH=CHCHOH), 126.17 (CH=CHCHOH), 108.05 (C(CH$_3$)$_2$), 79.76 (CHCHOTBS), 77.80 (CHCH$_2$), 72.92 (CHOTBS), 61.71 (CH$_2$OH), 30.87 (CH(CH$_3$)$_2$), 27.93 (CH$_3$ of *i*Pr), 25.97 (3 CH$_3$ of *t*But), 25.78 (CH$_3$ of *i*Pr), 22.23 (CH$_3$ of CH(CH$_3$)$_2$), 22.04 (CH$_3$ of CH(CH$_3$)$_2$), 18.31 (C quat of *t*Bu), -3.73 (CH$_3$ of TBS), -4.40 (CH$_3$ of TBS). HRMS (ESI+): m/z calcd for $C_{18}H_{36}O_4Si$ [M+Na]$^+$: 367.2281; found: 367.2322.

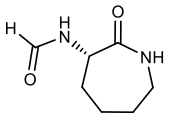


(R, E)-1-((4R,5S)-5-(hydroxymethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-4-methylpent-2-en-1-ol 4.32

Colourless oil, R*f* = 0.32 (ETP: Et$_2$O 1:2), developed with Hanessian stain. [α]$^{22}_D$= + 23.83 (c = 1.19, CH$_3$Cl)
^{1}H NMR (300 MHz, CDCl$_3$, 25 °C, TMS): δ = 5.77 (dd, J = 15.6, 6.5 Hz, 1H, CH=CHCHOTBS), 5.54 (ddd, J = 15.6, 6.2, 1.0 Hz, 1H, CH=CHCHOTBS), 4.37 – 4.23 (m, 2H, CHOTBS and CHCH$_2$), 4.04 (dd, J = 8.0, 5.8 Hz, 1H, CHCHOTBS), 3.92 – 3.71 (m, 2H, CH$_2$OH), 3.18 (broad s, 1H, OH), 2.99 (broad s, 1H, OH), 2.46 – 2.21 (m, 1H, CH(CH$_3$)$_2$), 2.17 (s, 3H), 1.43 (s, 3H, CH$_3$ of

*i*Pr), 1.36 (s, 3H, CH_3 of *i*Pr), 1.02 (d, J = 6.7 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS); δ = 141.19 (CH=CHCHOH), 126.17 (CH=CHCHOH), 108.50 ($C(CH_3)_2$), 79.81 (CHCHOH), 77.54 ($CHCH_2$), 70.81 (CHOH), 61.01 (CH_2OH), 30.95 ($CH(CH_3)_2$), 27.91 (CH_3 of *i*Pr), 25.50 (CH_3 of *i*Pr), 22.35 (CH_3 of $CH(CH_3)_2$), 22.25 (CH_3 of $CH(CH_3)_2$).). *IR* ν (cm^{-1}) = 3345; 2959; 2935; 2871; 1460; 1376; 1245; 1218; 1167; 1132; 1070; 1038; 971; 920; 886; 854; 794. HRMS (ESI+): m/z calcd for $C_{11}H_{12}O_4$ $[M+Na]^+$: 253.1416; found: 253.1398.

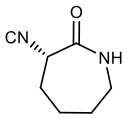
4.4.2.4 Synthesis of isocyanide



(S)-N-(2-oxoazepan-3-yl)formamide 4.34

To a solution of L -(−)-α-amino-ε-caprolactam hydrochloride **4.33** (500 mg, 3.04 mmol) in dry CH_2Cl_2 (15.2 mL) under nitrogen atmosphere, Et_3N (591 µL, 4.25 mmol) was added. Then, formic acid (183 µL, 4.86 mmol) and DCC (277 mg, 4.25 mmol) were added e to the solution at 0°C and the reaction was stirred at room temperature for 19 h. The mixture was filtered through a pad of celite, washing with CH_2Cl_2 and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography on silica gel (AcOEt + 2% MeOH and AcOEt + 10% MeOH) to afford **4.34** as a white amorphous solid (428 mg, 90% yield).

4.34, white amorphous solid, R*f* = 0.42 (DCM: MeOH 9:1), developed with Hanessian stain. $[\alpha]^{22}_D$= - 8.48 (c = 1.01, CH_3Cl).
^{1}H NMR (300 MHz, CDCl3, 25 °C, TMS): δ = 8.20 (s, 1H, CHO), 7.14 (s, 1H, NH), 6.50 (s, 1H, NH), 4.61 (dd, *J*= 11.1, 6.2 Hz, 1H, CHNH), 3.33 –3.24 (m, 2H, CH_2NH), 2.19 –2970 (m, 2H, CH_2 of caprolactam), 1.94 –1.75 (m, 2H, CH_2 of caprolactam), 1.61–1.32 (m, 2H, CH_2 of caprolactam). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS); δ = 175.1 (C=O), 160.2 (CHO), 51.0 (CHNH), 42.1 (CH_2NH), 31.5 (CH_2 of caprolactam), 28.8 (CH_2 of caprolactam), 27.9 (CH_2 of caprolactam). *IR* ν (cm^{-1}) = 3268; 3089; 2972; 2912; 2866; 2850; 1695; 1628; 1517; 1482; 1437; 1381; 1370; 1361; 1335; 1316; 1292; 1278; 1222; 1212; 1122; 1092; 1057; 1043; 978; 946; 910; 851; 835; 804; 759; 726; 660. *HRMS* (ESI+): m/z calcd for $C_7H_{12}N_2O_2$ $[M+Na]^+$: 179.9797: found: 179.9856.

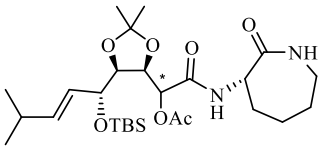


(S)-3-isocyanoazepan-2-one 4.35

Compound **4.34** (250 mg, 1.60 mmol) was dissolved in dry CH_2Cl_2 (8 mL) under nitrogen atmosphere. Et_3N (1.05 mL, 7.52 mmol) and $POCl_3$ (228 µL, 2.40 mmol) were added dropwise at -30°C. The reaction was stirred for 1.30 h at -30° C. Then it was quenched with 40 mL of $NaHCO_3$ (sat.) extracted with AcOEt (3 x 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography on silica gel (ETP: AcOEt 1: 5) to afford **4.35** as a white amorphous solid (190 mg, 86% yield).

4.35, white amorphous solid, R*f* = 0.63 (DCM: MeOH 9:1), developed with Hanessian stain. $[\alpha]^{24}_D$= -11.2 (c = 1.03, CH_3Cl)
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = (s, 1H, NH), 4.49 (d, J= 9.6 Hz, 1H, CHNC), 3.47 –3.31 (m,1H, CH_2NH), 3.12 (ddd, J= 15.5, 10.1, 5.7Hz, 1H, CH_2NH), 2.21 –1.96 (m, 3H, H of caprolactam), 1.85 –1.68 (m, 2H, H of caprolactam), 1.66 – 1.48 (m, 1H, H of caprolactam). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 170.0 (C=O), 159.4 (NC), 57.7 (CHNC), 41.5 (CH_2NH), 31.2 (CH_2 of caprolactam), 28.4 (CH_2 of caprolactam), 26.7 (CH_2 of caprolactam). *IR* ν (cm^{-1}) = 3328; 3223; 3099; 2992; 2948; 2925; 2858; 2148; 1670; 1478; 1466; 1436; 1428; 1359; 1331; 1323; 1291; 1274; 1248; 1111; 1092; 1075; 1038; 1015; 964; 944; 885; 823; 789; 776; 687. *HRMS* (ESI+): m/z calcd for $C_7H_{10}N_2O_2$ $[M+Na]^+$: 161.0691; found: 161.0634.

4.4.2.5 Synthesis of Passerini product



(R)-1-((4R,5S)-5-((R,E)-1-((tert-butyldimethylsilyl) oxy)-4-methylpent-2-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-oxo-2-(((S)-2-oxoazepan-3-yl) amino) ethyl acetate 4.37

Swern oxidation

Aldehyde **4.36** was prepared employing the same procedure of **4.19** from alcohol **4.20** (65 mg, 0.19 mmol). R*f* = 0.81 (ETP: Et_2O 3:1), developed with Hanessian stain.

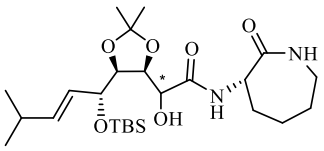
Passerini reaction

To a solution of crude aldehyde **4.36** (0.19 mmol) in iPr_2O (500 μL, dried over molecular sieves 4Å) under nitrogen atmosphere, acetic acid (22 μL, 0.39 mmol) and isocyanide **4.32** (53 mg, 0.39 mmol) were added. The reaction was stirred at room temperature for 21 h. The reaction solvent was removed by evaporation under reduced pressure and the residue was filtered on silica gel (ETP: AcOEt 3:4) and directly used for the next reaction.

4.37_*anti* pale yellow solid; R*f* = 0.57 (ETP: AcOEt 1:5), developed with Hanessian stain. $[\alpha]^{19}_D$= -22.9 (c = 0.98, $CHCl_3$).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.33 (d, J = 5.6 Hz, 1H, NHCH), 6.07 (t, J = 6.1 Hz, 1H, $NHCH_2$), 5.67 (dd, J = 15.6, 6.0 Hz, 1H, CH=CHCHOTBS), 5.54 (ddd, *J* = 15.6, 7.9, 1.0 Hz, 1H, CH=CHCHOTBS), 5.23 (d, J = 7.4 Hz, 1H, CHOAc), 4.64 (dd, J = 7.8, 4.5 Hz, 1H, CHOTBS), 4.59 – 4.46 (m, 1H, CHNH), 4.40 (dd, J = 7.3, 6.1 Hz, 1H, CHCHOAc), 4.09 (dd, J = 5.9, 4.5 Hz, 1H, CHCHOTBS), 3.36 – 3.12 (m, 2H, CH_2NH), 2.40 – 2.20 (m, 1H, $CH(CH_3)_2$), 2.12 (s, 3H, CH_3 of OAc), 2.04 – 1.56 (m, 6H, 3 CH_2 of caprolactam), 1.43 (d, J = 1.0 Hz, 3H, CH_3 of *i*Pr), 1.32 (s, 3H, CH_3 of *i*Pr), 1.01 (dd, J = 6.7, 1.0 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$), 0.87 (s, 9H, 3 CH_3 of *t*Bu), 0.11 (s, 3H, CH_3 of TBS), 0.05 (s, 3H, CH_3 of TBS). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 175.16 (C=ONH), 169.36 (C=ONH), 167.43 (OC=O), 141.55 (CH=CHCHOTBS), 126.82 (CH=CHCHOTBS), 108.68 ($C(CH_3)_2$), 80.73 (CHCHOTBS), 76.16 (CHCHOAc), 73.34 (CHOTBS), 72.83 (CHOAc), 52.30 (CHNH), 42.25 (CH_2NH), 31.13 (CH_2 of caprolactam), 30.85 ($CH(CH_3)_2$), 29.12 (CH_2

of caprolactam), 28.00 (CH_2 of caprolactam), 27.45 (CH_3 of *i*Pr), 26.08 (3 CH_3 of *t*Bu), 25.34 (CH_3 of *i*Pr), 22.27 (CH_3 of $CH(CH_3)_2$), 22.01 (CH_3 of $CH(CH_3)_2$), 21.01 (CH_3 of OAc), 18.46 (C quat of *t*Bu), - 3.69 (CH_3 of TBS), - 4.31 (CH_3 of TBS). *HRMS* (ESI+): m/z calcd for $C_{27}H_{48}N_2O_7Si$ $[M+Na]^+$: 563.3129; found: 563.3138.

4.37_*syn* pale yellow oil; R*f* = 0.60 (ETP: AcOEt 1:5), developed with Hanessian stain *^{1}H NMR* (300 MHz, CDCl3, 25 °C, TMS): δ = 7.36 (d, J = 7.0 Hz, 1H, NHCH), 5.93 – 5.77 (m, 1H, NHCH_2), 5.59 (dd, J = 15.5, 5.9 Hz, 1H, CH=CHCHOTBS), 5.43 (d, J = 1.2 Hz, 1H, CHOAc), 5.34 (ddd, J = 15.3, 8.2, 1.6 Hz, 1H, CH=CHCHOTBS), 4.62 (dd, J = 6.4, 1.2 Hz, 1H, CHCHOAc), 4.54 – 4.40 (m, 1H, CHNH), 4.11 (dd, J = 9.2, 6.2 Hz, 1H, CHCHOTBS), 4.02 – 3.88 (m, 1H, CHOTBS), 3.34 – 3.08 (m, 2H, CH_2NH), 2.43 – 2.24 (m, 1H, CH$(CH_3)_2$), 2.24 (s, 3H, CH_3 of OAc), 2.19 – 1.75 (m, 6H, 3 CH_2 of caprolactam), 1.32 (s, 3H, CH_3 of *i*Pr), 1.25 (s, 3H, CH_3 of *i*Pr), 1.02 (dd, J = 6.8, 4.2 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$), 0.89 (s, 9H, 3 CH_3 of *t*Bu) 0.09 (s, 3H, CH_3 of TBS), 0.03 (s, 3H, CH_3 of TBS). *HRMS* (ESI+): m/z calcd for $C_{27}H_{48}N_2O_7Si$ $[M+Na]^+$: 563.3129; found: 563.3138.



2-((4S,5S)-5-((R,E)-1-((tert-butyldimethylsilyl)oxy)-4-methylpent-2-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-hydroxy-N-((S)-2-oxoazepan-3-yl)-2l3-ethanamide 4.21

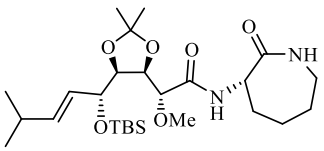
Triethylamine (200 μL) and H_2O (200 μL) were added to a stirred solution of **4.37** crude (0.19 mmol) in MeOH (1 mL). The mixture was stirred at room temperature for 2 d. When the reaction was completed (monitored by HPLC analysis), the solvent was removed by evaporation under reduced pressure and the residue was purified by flash column chromatography on silica gel (Et_2O: ETP 20:1) to afford **4.21_*anti*** and **4.21_*syn*** as a mixture of diastereoisomers (d.r. = 80: 20 calculated by HPLC analysis on the crude product) (70 mg, 74% yield in three steps).

4.21_*anti*, pale yellow oil, R*f* = 0.35 ((Et_2O: ETP 20:1) developed with Hanessian stain. $[\alpha]^{20}_D$= +16.96 (c = 0.95, CH_3Cl).
^{1}H NMR (300 MHz, CDCl3, 25 °C,TMS): δ = 7.78 (d, J = 6.5 Hz, 1H, NHCH), 6.18 (broad s, 1H, NHCH_2), 5.62 (dd, J = 15.5, 6.6 Hz, 1H, CH=CHCHOTBS), 5.46 (dd, J = 15.5, 7.8 Hz, 1H, CH=CHCHOTBS), 4.63 – 4.51 (m, 3H, CHNH, OH and CHOTBS), 4.29 – 4.16 (m, 2H, CHCHOH and CHCHOH), 4.05 (t, J = 4.5 Hz, 1H, CHCHOTBS), 3.33 – 3.13 (m, 2H, CH_2NH), 2.41 – 2.18 (m, 1H, CH$(CH_3)_2$), 2.18 – 1.71 (m, 5H, H of caprolactam), 1.50 (s, 3H, CH_3 of *i*Pr), 1.48 – 1.32 (m, 1H, H of caprolactam), 1.32 (s, 3H, CH_3 of *i*Pr), 0.98 (dd, J = 6.8, 1.1 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$), 0.89 (s, 9H, 3 CH_3 of *t*Bu), 0.12 (s, 3H, CH_3 of TBS), 0.09 (s, 3H, CH_3 of TBS). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 175.49 (C=ONH), 170.58 (C=ONH), 142.02 (CH=CHCHOTBS), 125.92 (CH=CHCHOTBS), 108.47 (C$(CH_3)_2$), 80.83 (CHCHOTBS), 77.93 (CHCHOH), 73.61 (CHOTBS), 70.17 (CHCHOH), 52.15 (CHNH), 42.05 (CH_2NH), 31.43 (CH_2 of caprolactam), 30.85 (CH$(CH_3)_2$), 28.98 (CH_2 of caprolactam), 28.02 (CH_2 of caprolactam), 27.66 (CH_3 of

*i*Pr), 25.92 (3 CH_3 of *t*Bu), 25.62 (CH_3 of *i*Pr), 22.09 (CH_3 of $CH(CH_3)_2$), 21.93 (CH_3 of $CH(CH_3)_2$), 18.26 (C quat of *t*Bu), - 3.92 (CH_3 of TBS), - 4.35 (CH_3 of TBS). *IR* ν (cm^{-1}) = 3462; 3346; 2956; 2931; 2858; 1685; 1598; 1525; 1463; 1375; 1254; 1215; 1168; 1069; 1045; 974; 834; 800; 777; 667. *HRMS* (ESI+): m/z calcd for $C_{25}H_{46}N_2O_6Si$ $[M+Na]^+$:521.3023; found: 521.3018.

4.21_*syn*, pale yellow foam R*f* = 0.25 ((Et_2O: ETP 20:1) developed with Hanessian stain. $[\alpha]^{22}_D$= -33.85 (c = 1.20, CH_3Cl).
^{1}H NMR (300 MHz, CDCl3, 25 °C, TMS): δ = 7.98 (d, J = 7.0 Hz, 1H, NHCH), 6.16 (broad s, 1H, $NHCH_2$), 5.72 (dd, J = 15.6, 6.9 Hz, 1H, CH=CHCHOTBS), 5.38 (dd, J = 15.5, 6.1 Hz, 1H, CH=CHCHOTBS), 4.68 (t, *J* = 4.9 Hz, 1H, CHOTBS), 4.63 – 4.48 (m, 3H, CHNH, OH and CHCHOH), 4.29 (d, J = 2.2 Hz, 1H, CHCHOH), 4.17 (dd, J = 6.5, 4.4 Hz, 1H, CHCHOTBS), 3.40 – 3.13 (m, 2H, CH_2NH), 2.41 – 2.27 (m, 1H, $CH(CH_3)_2$), 2.16 – 1.65 (m, 5H, H of caprolactam), 1.61 – 1.51 (m, 1H, H of caprolactam) 1.49 (s, 3H, CH_3 of *i*Pr), 1.32 (s, 3H, CH_3 of *i*Pr) 0.99 (d, J = 6.7 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$), 0.92 (s, 9H, 3 CH_3 of *t*Bu), 0.14 (s, 3H, CH_3 of TBS), 0.11 (s, 3H, CH_3 of TBS). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 175.65 (C=ONH), 170.79 (C=ONH), 141.25 (CH=CHCHOTBS), 124.63 (CH=CHCHOTBS), 108.35 ($C(CH_3)_2$), 79.24 (CHCHOTBS), 77.77 (CHCHOH), 72.45 (CHOTBS), 71.50 (CHCHOH), 51.92 (CHNH), 42.28 (CH_2NH), 31.94 (CH_2 of caprolactam), 30.99 ($CH(CH_3)_2$), 29.18 (CH_2 of caprolactam), 28.17 (CH_2 of caprolactam), 26.35 (CH_3 of *i*Pr), 25.96 (3 CH_3 of *t*Bu), 25.49 (CH_3 of *i*Pr), 22.25 (CH_3 of $CH(CH_3)_2$), 22.22 (CH_3 of $CH(CH_3)_2$), 18.56 (C quat of *t*Bu), -4.22 (CH_3 of TBS), -4.73 (CH_3 of TBS). *HRMS* (ESI+): m/z calcd for $C_{25}H_{46}N_2O_6Si$ $[M+Na]^+$:521.3023; found: 521.3018.

4.4.2.6 Synthesis of 4-epi-Bengamide E

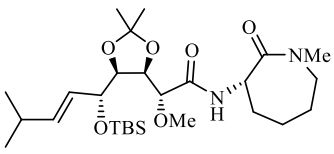


(R)-2-((4R,5S)-5-((R,E)-1-((tert-butyldimethylsilyl) oxy)-4-methylpent-2-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-methoxy-N-((S)-2-oxoazepan-3-yl) acetamide 4.38

A solution of **4.21_*anti*** (95 mg, 0.19 mmol) in dry THF (1 mL) under Ar atmosphere was cooled at -10°C. NaH (60% in silicon oil, 4.5 mg, 0.09 mmol) was added and the mixture was stirred for 30 minutes. Then MeI (18 µL, 0.19 mmol) was added and the reaction was stirred at -10°C. After 6 h other 0.6 eq of NaH were added and the reaction mixture was stirred for 18 h at -10°C. The reaction was quenched with 5 mL of NH_4Cl (sat) and extracted with DCM (3 x 5 mL). The combined organic layers were washed with LiCl (4 x 5 mL), dried (Na_2SO_4) and concentrated. From HPLC analysis, a starting material; product ratio of 45:55 was observed and no formation of side-products. Therefore, the reaction has been set up again using dry DMF as solvent (1 mL), NaH (8 mg, 0.19 mmol) and MeI (18 µL, 0.19 mmol). After 21 h the reaction was quenched as reported above and the starting material; product ratio checked by

HPLC analysis, resulting in 5:95. The residue was purified by flash column chromatography on silica gel (ETP: Et_2O 1:20) to give **4.38** (58 mg, 59%) as a colourless oil.

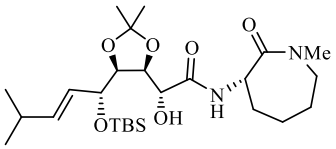
4.38, colourless oil, R*f* = 0.31 ((Et_2O + 2% AcOEt) developed with Hanessian stain. $[\alpha]^{22}_D$= + 6.24 (c = 1,73 CH_3Cl).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.53 (d, J = 6.2 Hz, 1H, NHCH), 6.02 (t, J = 6.5 Hz, 1H, $NHCH_2$), 5.72 – 5.49 (m, 2H, CH=CHCHOTBS and CH=CHCHOTBS), 4.67 – 4.52 (m, 2H, CHNH and CHOTBS), 4.25 – 4.18 (m, 1H, CHCHOTBS), 4.14 – 4.08 (m, 1H, $CHCHOCH_3$), 4.05 (d, J = 7.0 Hz, 1H, $CHOCH_3$), 3.34 (s, 3H, OCH_3), 3.30 – 3.15 (m, 2H, CH_2NH), 2.32 (m, 1H, $CH(CH_3)_2$), 2.15 – 1.76 (m, 6H, 3 CH_2 of caprolactam), 1.41 (s, 3H, CH_3 of *i*Pr), 1.29 (s, 3H, CH_3 of *i*Pr), 1.01 (dd, J = 6.7, 2.3 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$), 0.90 (d, J = 2.5 Hz, 9H, 3 CH_3 of *t*Bu), 0.10 (s, 3H, CH_3 of TBS), 0.07 (s, 3H, CH_3 of TBS). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 175.27 (C=ONH), 169.64 (C=ONH), 140.86 (CH=CHCHOTBS), 127.28 (CH=CHCHOTBS), 108.55 ($C(CH_3)_2$), 81.04 ($CHCHOCH_3$), 80.86 ($CHOCH_3$), 77.36 (CHCHOTBS), 73.33 (CHOTBS), 57.45 (OCH_3), 51.98 (CHNH), 42.23 (CH_2NH), 31.54 (CH_2 of caprolactam), 30.88 ($CH(CH_3)_2$), 29.11 (CH_2 of caprolactam), 28.02 (CH_2 of caprolactam), 27.22 (CH_3 of *i*Pr), 26.14 (3 CH_3 of *t*Bu), 25.27 (CH_3 of *i*Pr), 22.43 (CH_3 of $CH(CH_3)_2$), 22.04 (CH_3 of $CH(CH_3)_2$), 18.48 (C quat of *t*Bu), -3.48 (CH_3 of TBS), -4.25 (CH_3 of TBS). *IR* ν (cm^{-1}) = 3383; 3292; 2955; 2930; 2858; 1714; 1662; 1504; 1474; 1435; 1362; 1334; 1250; 1217; 1169; 1103; 1073; 1047; 1017; 972; 941; 899; 875; 834; 808; 776; 754; 666. *HRMS* (ESI+): m/z calcd for $C_{25}H_{46}N_2O_6Si$ $[M+Na]^+$:521.3023; found: 521.3018.



(R)-2-((4R,5S)-5-((R,E)-1-((tert-butyldimethyl silyl)oxy)-4-methylpent-2-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-methoxy-N-((S)-1-methyl-2-oxoazepan-3-yl)acetamide 4.40

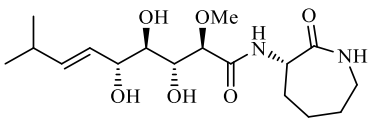
Colourless oil, R*f* = 0.38 ((Et_2O + 2% AcOEt) developed with Hanessian stain.
$[\alpha]^{22}_D$= + 1.60 (c = 0.63 CH_3Cl).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.61 (d, J = 6.0 Hz, 1H, NHCH), 5.78 – 5.46 (m, 2H, CH=CHCHOTBS and CH=CHCHOTBS), 4.70 (dd, J = 9.5, 6.3 Hz, 1H, CHNH), 4.56 (dd, J = 6.4, 4.3 Hz, 1H, CHOTBS), 4.20 (dd, J = 7.4, 6.2 Hz, 1H, CHCHOTBS), 4.08 (dd, J = 6.0, 4.2 Hz, 1H, $CHCHOCH_3$), 4.02 (d, J = 7.5 Hz, 1H, $CHOCH_3$), 3.61 (dd, J = 15.3, 11.6 Hz, 1H, CH_2NCH_3), 3.33 (s, 3H, OCH_3), 3.18 (dd, J = 15.0, 4.5 Hz, 1H, CH_2NCH_3), 3.04 (s, 3H, NCH_3), 2.31 (dt, J = 12.0, 6.0 Hz, 1H, $CH(CH_3)_2$), 2.12 (d, J = 12.7 Hz, 1H, H of caprolactam), 2.00 – 1.72 (m, 4H, 2 CH_2 of caprolactam), 1.42 (s, 3H, CH_3 of *i*Pr), 1.39-1.32 (m, 1H, H of caprolactam), 1.29 (s, 3H, CH_3 of *i*Pr), 1.01 (dd, J = 6.7, 1.9 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$), 0.90 (s, 9H, 3 CH_3 of *t*Bu), 0.10 (s, 3H, CH_3 of TBS), 0.07 (s, 3H, CH_3 of TBS). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS): δ = 172.77 (C=ONMe), 169.52 (C=ONH), 140.74

(CH=CHCHOTBS), 127.27 (CH=CHCHOTBS), 108.47 ($C(CH_3)_2$), 81.21 ($CHCHOCH_3$), 80.82 ($CHOCH_3$), 77.46 (CHCHOTBS), 73.30 (CHOTBS), 57.34 (OCH_3), 51.95 (CHNH), 50.52 (CH_2NMe), 36.04 (NCH_3), 31.72 (CH_2 of caprolactam), 30.89 ($CH(CH_3)_2$), 27.81 (CH_2 of caprolactam), 27.32 (CH_3 of *i*Pr), 26.79 (CH_2 of caprolactam), 26.15 (3 CH_3 of *t*Bu), 25.34 (CH_3 of *i*Pr), 22.45 (CH_3 of $CH(CH_3)_2$), 22.09 (CH_3 of $CH(CH_3)_2$), 18.51 (C quat of *t*Bu), -3.61 (CH_3 of TBS), -4.28 (CH_3 of TBS). *IR* ν (cm^{-1}) = 3388; 2955; 2930; 2858; 2246; 1648; 1495; 1461; 1403; 1381; 1370; 1339; 1251; 1214; 1157; 1138; 1102; 1075; 1047; 1016; 973; 910; 879; 834; 808; 777; 729; 646. *HRMS* (ESI+): m/z calcd for $C_{27}H_{50}N_2O_6Si$ $[M+Na]^+$:549.3336; found: 549.3299.



(R)-2-((4S,5S)-5-((R,E)-1-((tert-butyldimethylsilyl) oxy)-4-methylpent-2-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-hydroxy-N-((S)-1-methyl-2-oxoazepan-3-yl)acetamide 4.39

Colourless oil, R*f* = 0.33 (Et_2O + 2% AcOEt) developed with Hanessian stain.
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.87 (d, J = 5.8 Hz, 1H, NHCH), 5.63 (dd, J = 15.3, 6.8 Hz, 1H, CH=CHOTBS), 5.55 – 5.41 (m, 1H, CH=CHOTBS), 4.74 – 4.62 (m, 1H, CHNH), 4.62 – 4.54 (m, 1H, CHOTBS), 4.53 (d, *J* = 3.6 Hz, 1H, CHOH), 4.21 (dd, J = 7.2, 4.4 Hz, 2H, CHCHOTBS and OH), 4.06 (t, J = 4.7 Hz, 1H, CHCHOH), 3.65 – 3.52 (m, 1H, CH_2NCH_3), 3.25 – 3.10 (m, 1H, CH_2NCH_3), 3.03 (s, 3H, NCH_3), 2.42 – 2.14 (m, 1H, $CH(CH_3)_2$), 2.19 – 1.67 (m, 4H, H of caprolactam), 1.52 (s, 3H, CH_3 of *i*Pr), 1.48 – 1.36 (m, 2H, H of caprolactam) 1.33 (s, 2H, CH_3 of *i*Pr), 1.00 (dd, J = 6.7, 1.8 Hz, 6H, 2 CH_3 of $CH(CH_3)_2$), 0.90 (s, 9H, 3 CH_3 of *t*Bu), 0.13 (s, 3H, CH_3 of TBS), 0.11 (s, 3H, CH_3 of TBS). *HRMS* (ESI+): m/z calcd for $C_{24}H_{48}N_2O_6Si$ $[M+Na]^+$:535.318; found: 535.3211.

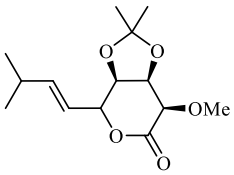


(2R,3R,4R,5R,E)-3,4,5-trihydroxy-2-methoxy-8-methyl-N-((S)-2-oxoazepan-3-yl)non-6-enamide 4.22

Compound **4.38** (38 mg, 0.07 mmol) was solubilized in THF (400 µL) and HCl 1M (800 µL) was added. The reaction was stirred for 6 h at room temperature. The reaction was quenched with $NaHCO_3$ (sat.) (pH =9) and extracted with AcOEt (3 x 5 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography on silica gel (AcOEt + 2% MeOH and AcOEt: MeOH 9:1) to afford **4.22** as a pale yellow foam (9.4 g, 35% yield).

4.22, pale yellow foam, R*f* = 0.42 (AcOEt + 2% MeOH) developed with Hanessian stain. $[\alpha]^{22}_D$= +34.5 (c = 0.30, CH_3Cl).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.63 (d, J = 7.0 Hz, 1H, NHCH), 6.22 (t, J = 6.1 Hz, 1H, $NHCH_2$), 5.78 (ddd, J = 15.6, 6.4, 0.9 Hz, 1H, CH=CHCHOH), 5.55

(ddd, J = 15.6, 7.2, 1.3 Hz, 1H, CH=C<u>H</u>CHOH), 4.59 (dd, J = 10.3, 7.0 Hz, 1H, C<u>H</u>NH), 4.26 (t, J = 5.26, 1H CH=CHC<u>H</u>OH), 4.07 (d, J = 3.3 Hz, 1H, C<u>H</u>OCH$_3$), 4.07 – 3.95 (m, 1H, C<u>H</u>CHOCH$_3$), 3.77 (d, J = 4.7 Hz, 1H, CH(OH)CH(O<u>H</u>)CH(OH)), 3.62 (d, J = 8.2 Hz, 1H, CH(OH)C<u>H</u>(OH)CH(OH)), 3.49 (s, 3H, OCH$_3$), 3.49 (s, 1H, CH(O<u>H</u>)CHOCH$_3$), 3.39 – 3.18 (m, 2H, C<u>H$_2$</u>NH), 2.77 (broad s, 1H CH=CHCHO<u>H</u>,), 2.33 (dq, J = 13.4, 6.8 Hz, 1H, C<u>H</u>(CH$_3$)$_2$), 2.13 – 1.98 (m, 3H, H of caprolactam), 1.96 – 1.72 (m, 1H, H of caprolactam), 1.64 – 1.31 (m, 2H, H of caprolactam)., 1.01 (d, J = 6.8 Hz, 6H, 2 CH$_3$ of CH(CH$_3$)$_2$). *^{13}C NMR* (75 MHz, CDCl$_3$, 25 °C, TMS): δ = 175.25 (C=ONH), 170.68 (C=ONH), 141.76 (<u>C</u>H=CHCHOH), 125.25 (CH=<u>C</u>HCHOH), 82.60 (<u>C</u>HOCH$_3$), 74.65 (CH=CH<u>C</u>HOH), 74.01 (CH(OH)<u>C</u>H(OH)CH(OH)), 72.99 (<u>C</u>HCHOCH$_3$), 58.88 (O<u>C</u>H$_3$), 52.45 (<u>C</u>HNH), 42.23 (<u>C</u>H$_2$NH), 30.99 (<u>C</u>H(CH$_3$)$_2$), 30.76 (<u>C</u>H$_2$ of caprolactam), 28.94 (<u>C</u>H$_2$ of caprolactam), 28.09 (<u>C</u>H$_2$ of caprolactam), 22.47 (CH$_3$ of CH(CH$_3$)$_2$), 22.34 (CH$_3$ of CH(CH$_3$)$_2$). *IR* ν (cm^{-1}) = 3334; 2956; 2930; 2868; 1639; 1519; 1483; 1437; 1361; 1334; 1291; 1261; 1066; 973; 943; 893; 800; 720. *HRMS* (ESI+): m/z calcd for C$_{17}$H$_{30}$N$_2$O$_6$ [M+Na]$^+$:381.2002; found: 381.1993

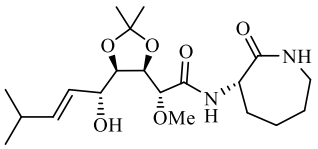


(3aR,7S,7aR)-7-methoxy-2,2-dimethyl-4-((E)-3-methylbut-1-en-1-yl)tetrahydro-6H-[1,3]dioxolo[4,5-c]pyran-6-one 4.41

Colourless oil, R*f* = 0.81 ((AcOEt + 2% MeOH) developed with Hanessian stain.

^{1}H NMR (300 MHz, CDCl$_3$, 25 °C, TMS): δ = 5.82 (ddd, J = 15.8, 6.8, 2.1 Hz, 1H, C<u>H</u>=CHCHO), 5.45 (ddd, J = 15.9, 4.1, 1.3 Hz, 1H, CH=C<u>H</u>CHO), 5.02 (d, J = 1.8 Hz, 1H, CH=CHC<u>H</u>O), 4.82 (dd, J = 7.6, 3.5 Hz, 1H, C<u>H</u>CHOCH$_3$), 4.56 (dd, J = 7.6, 1.1 Hz, 1H, CH=CHCHC<u>H</u>), 4.07 (d, J = 3.5 Hz, 1H, C<u>H</u>OCH$_3$), 3.63 (s, 3H, OCH$_3$), 2.37 (dq, J = 13.5, 6.8 Hz, 1H, C<u>H</u>(CH$_3$)$_2$), 1.50 (s, 3H, CH$_3$ of *i*Pr), 1.36 (s, 3H, CH$_3$ of *i*Pr), 1.02 (d, J = 6.7 Hz, 6H, 2 CH$_3$ of CH(CH$_3$)$_2$).

^{13}C NMR (75 MHz, CDCl$_3$, 25 °C, TMS): δ = 168.57 (C=O), 142.67 (<u>C</u>H=CHCHO), 121.32 (CH=<u>C</u>HCHO), 111.00 (<u>C</u>H(CH$_3$)$_2$), 79.67 (CH=CH<u>C</u>HO), 76.16 (<u>C</u>HOCH$_3$), 75.71 (CH=CHCH<u>C</u>H), 74.77 (<u>C</u>HCHOCH$_3$), 59.88 (OCH$_3$), 31.29 (<u>C</u>H(CH$_3$)$_2$), 26.24 (CH$_3$ of *i*Pr), 24.41 (CH$_3$ of *i*Pr), 22.05 (CH$_3$ of CH(CH$_3$)$_2$), 21.96 (CH$_3$ of CH(CH$_3$)$_2$). *HRMS* (ESI+): m/z calcd for C$_{14}$H$_{22}$O$_5$ [M+Na]$^+$:293.1365; found: 293.1349



(R)-2-((4R,5R)-5-((R,E)-1-hydroxy-4-methylpent-2-en-1-yl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2-methoxy-N-((S)-2-oxoazepan-3-yl)acetamide 4.43

Colourless oil, R*f* = 0.38 (AcOEt + 2% MeOH) developed with Hanessian stain.

^{1}H NMR (300 MHz, CDCl$_3$, 25 °C, TMS): δ = 7.51 (d, J = 6.3 Hz, 1H, N<u>H</u>CH), 6.04 (t, J = 6.0 Hz, 1H, N<u>H</u>CH$_2$), 5.83 (dd, J = 15.6, 6.5 Hz, 1H, C<u>H</u>=CHCHOH), 5.56 (dd, J = 15.6, 5.3 Hz, 1H, CH=CHC<u>H</u>OH), 4.62 (dd, J = 10.5, 7.2 Hz, 1H, C<u>H</u>NH), 4.33

(dd, J = 7.9, 5.1 Hz, 1H, C<u>H</u>CHOCH$_3$), 4.24 – 4.15 (m, 1H, C<u>H</u>OH), 4.03 (dd, J = 9.0, 5.2 Hz, 1H, C<u>H</u>CHOH), 3.92 (d, J = 8.0 Hz, 1H, C<u>H</u>OCH$_3$), 3.68 (d, J = 3.4 Hz, 1H, OH), 3.45 (s, 3H, OCH$_3$), 3.29 (dd, J = 10.3, 5.0 Hz, 2H, C<u>H$_2$</u>NH), 2.45 – 2.23 (m, 1H, C<u>H</u>(CH$_3$)$_2$), 2.26 – 1.76 (m, 5H, H of caprolactam), 1.57 – 1.41 (m, 1H, H of caprolactam) 1.48 (s, 3H, CH$_3$ of *i*Pr), 1.32 (s, 3H, CH$_3$ of *i*Pr), 1.02 (d, J = 6.7 Hz, 6H, 2 CH$_3$ of CH(CH$_3$)$_2$). ^{13}C NMR (75 MHz, CDCl$_3$, 25 °C, TMS): δ = 175.16 (C=ONH), 168.57 (C=ONH), 139.85 (<u>C</u>H=CHCHOH), 126.01 (CH=<u>C</u>HCHOH), 109.46 (<u>C</u>(CH$_3$)$_2$), 81.24 (CH<u>C</u>HOCH$_3$), 81.04 (<u>C</u>HCHOCH$_3$), 77.46 (<u>C</u>HCHOH), 69.21 (<u>C</u>HOH), 58.36 (O<u>C</u>H$_3$), 52.20 (<u>C</u>HNH), 42.30 (<u>C</u>H$_2$NH), 31.43 (<u>C</u>H$_2$ of caprolactam), 30.99 (<u>C</u>H(CH$_3$)$_2$), 29.06 (<u>C</u>H$_2$ of caprolactam), 28.01 (<u>C</u>H$_2$ of caprolactam), 27.93 (CH$_3$ of *i*Pr), 25.64 (CH$_3$ of *i*Pr), 22.51 (CH$_3$ of CH(CH$_3$)$_2$), 22.39 (CH$_3$ of CH(CH$_3$)$_2$). *HRMS* (ESI+): m/z calcd for $C_{20}H_{34}N_2O_6$ $[M+Na]^+$:421.2315; found: 421.2310

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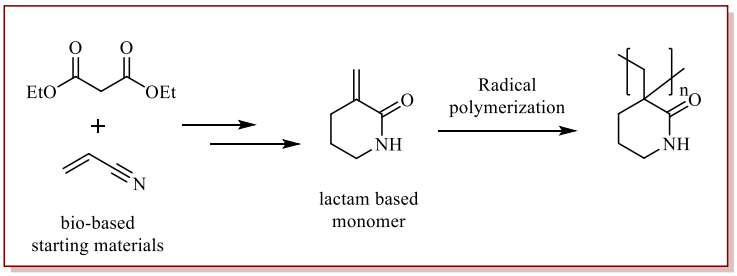
Part B

Bio-based building blocks in materials science

In this section, the second part of my doctoral research, carried out at the University of Stellenbosch (South Africa), will be discussed.

In the time period occurring between 27th January 2019 and 27th July 2019, I had the pleasure of working in the group of Prof. Bert Klumperman and Dr. Rueben Pfukwa, at Stellenbosch University, in South Africa.

During the internship, I was assigned to a project dedicated to the synthesis of a lactam- based monomer derived from bio-based starting materials and its subsequent polymerization.



This work was included in the Project "Synthesis, Characterization, Structure and Properties of Novel Biodegradable Polyesters (BIODEST)" that is funded under the RISE (Research and Innovation Staff Exchange) Scheme (H2020-MSCA-RISE-2017-778092).

CHAPTER 5.
Synthesis and polymerization of 3M2Pip

5.1 Introduction

5.1.1 BIODEST Programme

BIODEST (Synthesis, Characterization, Structure and Properties of Novel Biodegradable Polyesters)[1] is a RISE (Research and Innovation Staff Exchange) project that promotes student and staff exchanges between European Research and non-EU Research partners and collaborators with the aim to improve skills and knowledge in the topic of novel biodegradable polyesters. The programme involves nine academic organizations (University of the Basque Country in Spain, University of Mons in Belgium, University of Genova in Italy, University of Warwick in U.K., University of Tulane in USA, Chinese Academy of Science in China, Simon Bolivar University in Venezuela, University of Stellenbosch in South Africa and Yamagata University in Japan) and a non-academic organization "Ma+D - Waste Valorization Engineering".

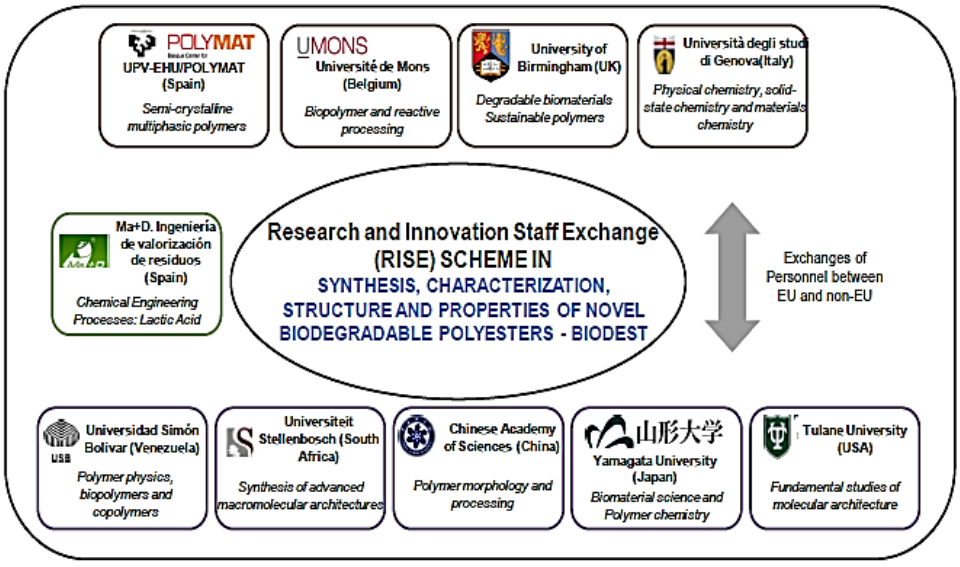


Figure 5.1 BIODEST consortium

In detail, BIODEST project has as its objective the green production of novel sustainable plastics. It aims to contribute to preserve our environment by synthesizing or formulating bio-based and biodegradable polyesters (or copolyesters) that can substitute traditional plastics in commodity applications (like the ubiquitous plastic bags or water bottles) or biomedical applications (like drug delivery vehicles, devices and scaffolds for tissue engineering).

The research activity in BIODEST project is divided into five Work Packages illustrated in *Figure 5.2* with the involved partners for each package.

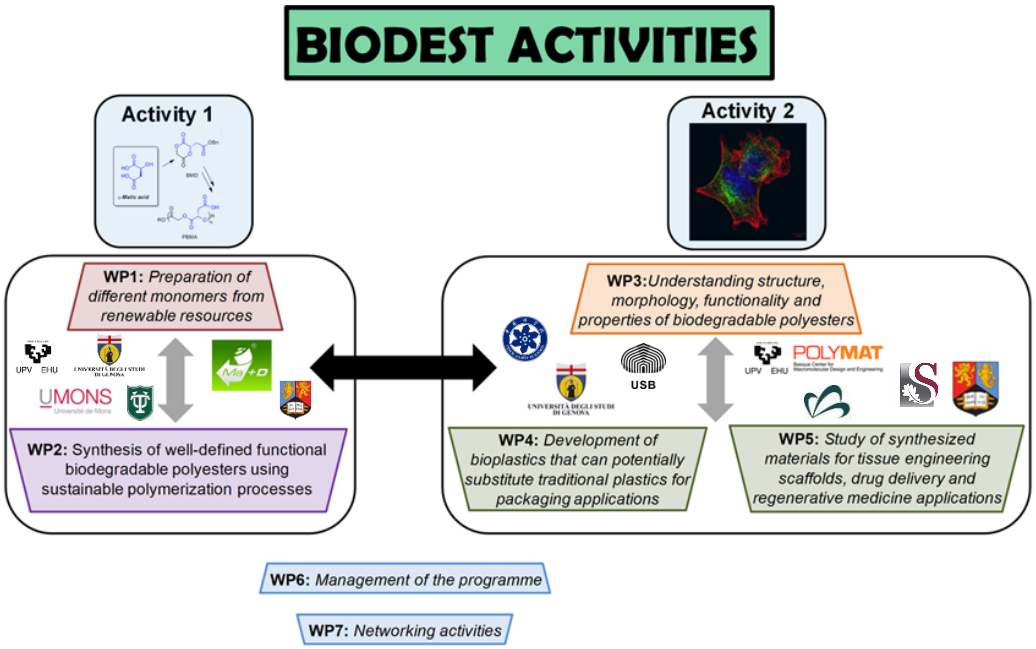


Figure 5.2 Work packages in BIODEST Project

The work of Free Radical group in the project belongs to Work Package 5 (Study of synthesized materials for tissue engineering scaffolds, drug delivery and regenerative medicine applications). The general objective of this task involves the study of self-assembly of synthesized polymers for drug delivery applications and the production of polyesters and copolyesters that can be applied in the tissue engineering field. In this context, the role of Klumperman's groups in the RISE project is summarized in the following three points: 1) Synthesis of lactam-based polymers-polyester block copolymers drug delivery vehicles. 2) Development of antimicrobial nanofiber mats from functional polyesters. 3) Application of degradable polyesters in advanced macromolecular architectures.

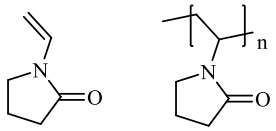
Related to this, the project in which I was involved during my secondment, focused the synthesis of degradable bio-based block copolymers composed of lactam-based polymers and of polyesters, that can find application in drug delivery polymer materials.

5.1.2 Lactam based polymers

Lactams-based polymers present some important features, such as water solubility, biocompatibility, hydrogen bonding and coordination capacity that make them important in various application in the food, pharmaceutical and cosmetic industries[2]. These polymers present a repeat unit with a highly polar cyclic amide, a

group capable of forming hydrogen bonds, whereas the methylene group of the backbone have a non- polar character. As a result, lactam-based polymers are generally soluble in water and in a range of organic solvent.

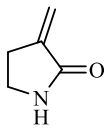
Most literature reports utilise *N*-substituted vinyl pyrrolidone, the preeminent example being poly(*N*-vinylpyrrolidone) (PVP), the polymer of *N*-vinylpyrrolidone (NVP) (*Scheme 5.1*). The high versatility of PVP can be explained by its diverse properties including its solubility in water and in a broad range of liquid media, high chemical and thermal resistance, and unique wetting, binding, and film-forming properties. Thanks to biocompatibility, absence of toxicity and high capacity to form interpolymer complexes, PVP is widely used for designing materials for different applications, such as biomaterials for medical and nonmedical uses.



Scheme 5.1 NVP and PVP

A much less reported system of pyrrolidone functional polymers is based on monomers in which the vinyl/methylene group is directly attached to the pyrrolidone ring in position 3 (*Scheme 5.2,* 3-methylene-2- pyrrolidone (3M2P) based monomers). Even though the 3M2P class of polymers did exhibit very interesting properties, regard to its aqueous solubility, non-toxicity, superior thermal properties and possibility of copolymerization, very few reports have been dedicated to the fundamental characterization of 3M2P-based polymers.

In a previous work, conducted in Klumperman's group, the synthesis and polymerization (by free radical and RAFT mechanism) of 3M2P monomer[3] and the thermoresponsive behaviour of poly(3M2P) polymers had been studied[4]. Then, the application of 3M2P derivatives as commercial low dosage hydrate inhibitors in the oil and gas industry has been evaluated[5]. Finally, the feasibility of using poly(3M2P) based block copolymers in biomedical applications has been investigated.

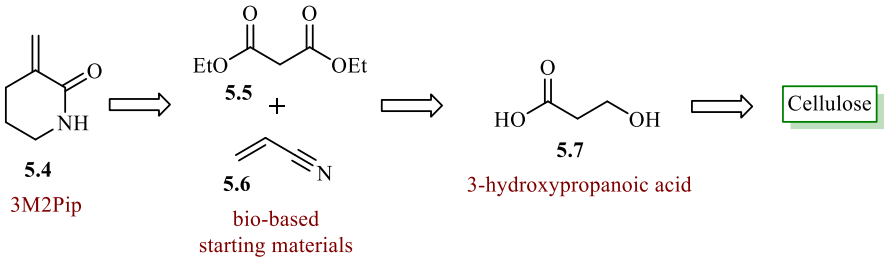


Scheme 5.2 3M2P

Due to the importance of these kind of polymers, i.e. water soluble, biocompatible, it might be interesting to synthesize new 3M2P- based monomers and study their polymerization and properties.

5.1.3 Research plan

Among the possible lactam-based monomers, the work was carried out on 3-methylene-2-piperidone (3M2Pip), which can be obtained from the reaction between diethyl malonate and acrylonitrile (*Scheme 5.3*). These are considered bio-based compounds since they derived from 3-hydroxypropionic acid (3-HP), a cellulose C3 building blocks derivative[6].



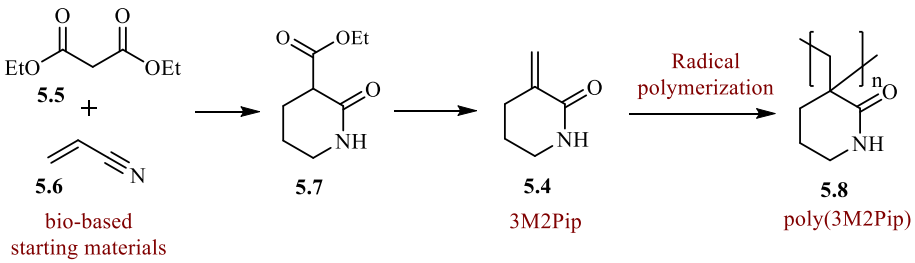
Scheme 5.3 3M2Pip from bio-based starting materials

Malonic acid is a standard component for many products and processes in the petrochemical, pharmaceutical, and cosmetics industries. It is used as a precursor in speciality polyesters and polymers and it is used to control acidity by either acting as an excipient in pharmaceutical drug formulations or as a natural preservative additive for foods[7]. Acrylonitrile is a very important, versatile monomer and chemical intermediate, with many commercial applications. It is used in the production of fibres (carpets, draperies, fabrics, nylons), resins (polyacrylates automotive industry), and many speciality products (paints, caulking materials, cosmetics, etc.).

Considering the importance of this bio-based lactam moiety, we have a long term interest in the synthesis and polymerization of 3-methylene-2-piperidone (3M2Pip) monomers, targeting the synthesis of well-defined hydroxyl end functional P(3M2Pip), i.e. HO-P(3M2Pip). Once obtained, HO-P(3M2Pip) can be used as a macro-initiator for the synthesis of degradable polyester second blocks, applicable in drug delivery polymer materials.

In earlier work, in the Klumperman group, 3M2Pip was synthesized by the colleague Dr. Ingrid Heyns (more details about her work are discussed in *Paragraphs 5.2.1*) and subsequently polymerized via FRP; however, monomer synthesis was achieved in low yields, and was not optimized. Therefore, this project mainly focused on two objectives, shown in *Scheme 5.4*:

1) Optimization of the synthesis of 3M2Pip monomer, starting from intermediate **5.7**. This, in particular, was the main part of this project.
1) Preliminary investigation into the polymerization of 3M2Pip via conventional free radical mechanism.



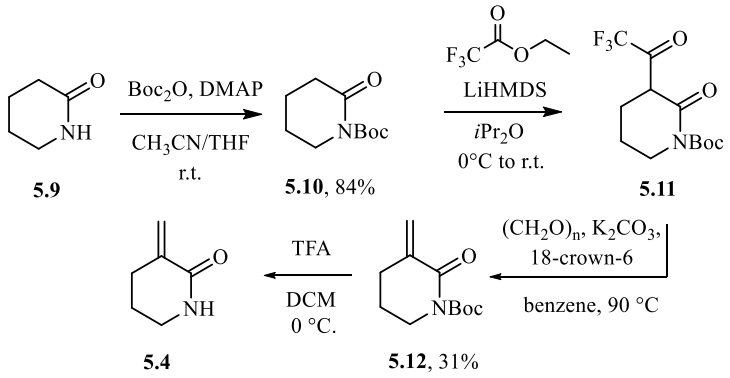
Scheme 5.4 Research plan

5.2 Results and Discussion

In the following paragraphs the results obtained and the problem encountered will be described. Firstly (*Paragraph 5.2.1*) the optimization of the synthesis of the desired monomer will be discussed, focusing on the application of a different synthetic strategy respect to the previously exploited by the group. Then, preliminary attempts of the polymerization reaction to obtain poly(2M3P) polymer will be described (*Paragraph 5.2.2*).

5.2.1 Optimization of the synthesis of 3M2Pip

In the work previously conducted in the Klumperman group[8] 3M2Pip was synthesized starting from commercially available 2-piperidone **5.9** in a 4 steps sequence (*Scheme 5.5*).



Scheme 5.5 Previous synthesis of 3-methylene-2-piperidone

N-Boc protection of 2- piperidone **5.9** was performed in acetonitrile/THF, with DMAP as catalyst, giving the product **5.10** in a good yield. Subsequently, *N*-Boc-2-piperidone was treated with the base, LiHDMS, to form an enolate derivative which reacted with ethyl trifluoroacetate at position 3 of the ring. The successive reaction involved reacting the (trifluoro)-acetylated derivatives **5.11** with paraformaldehyde in

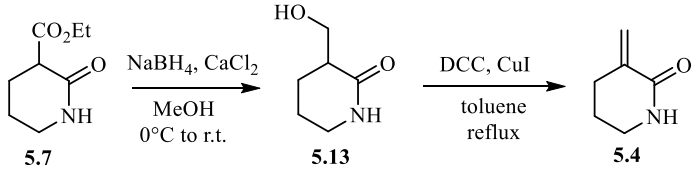
order to produce the 3-methylene derivatives **5.12**. The methylenation by the release of oxalate was also attempted for the *N*-Boc protected 2-piperidone, however, the release of trifluoroacetate gave higher yields. Finally, the *N*-Boc deprotection was performed with trifluoroacetic acid in DCM to afford the final product **5.4**.

This approach gave the desired product in four steps, with two purification steps (of compounds **5.10** and **5.12**), starting from commercially available 2-piperidone **5.9**. However, the main problems of this approach were the low yields of the two last steps. Moreover, the reaction involves the use of protecting groups with consequently more steps and the use of solvents such as benzene, something that is preferable to avoid. The main objective during the previous work was to obtain the monomer in enough yield in order to convert to the polymer at a later step. Consequently, no much time was spent on optimizing the monomer synthesis steps and only the approach in *Scheme 6.5* was attempted.

For this reason, the first aim accomplished during this investigation entailed the optimization of this synthesis. Initially, all the reactions were performed on a small scale (milligrams scale) in order to optimize the procedures. Then, since the objective of this part of the work was to obtain enough monomer to later perform polymerization reactions, a scale-up of the procedures was performed (grams scale).

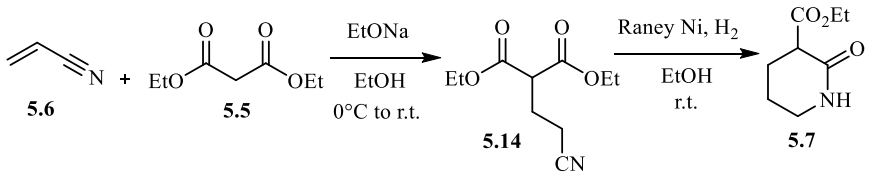
After a brief literature research, it was decided to completely change the synthetic strategy in order to reduce the synthetic steps.

Among the different methodologies to introduce a double bond, an interesting approach involves a two steps sequence that could be applied in our case. As shown in *Scheme 5.6*, the α-methylene lactam **5.4** can be easily prepared by direct dehydration with *N,N'*-Dicyclohexylcarbodiimide (DCC) in the presence of catalytic amounts of cuprous iodide of the corresponding alcohol **5.13** obtained starting from the six-member ring bearing ester moiety **5.7**[9].



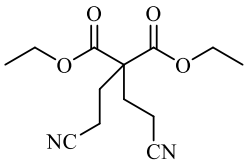
Scheme 5.6 New synthesis of 3-methylene-2-piperidone

This sequence affords the desired product in only two steps and there is no necessity of protecting groups. Starting material **5.7** is commercially available but it can be also prepared from acrylonitrile and diethyl malonate by Michael addition reaction and subsequent reduction (*Scheme 5.7*)[10].



Scheme 5.7 Synthesis of starting material

Initially, the synthesis of compound **5.7** was attempted, following the above procedure. Michael addition was performed with sodium ethoxide, prepared in situ from Na and dry EtOH, at room temperature under an argon atmosphere. During the reaction, the formation of a side-product deriving from the double attack to the malonate can occur (compound. **5.15**, *Scheme 5.8*)



5.15

Diethyl 2,2- bis(2-cyanoethyl) malonate)

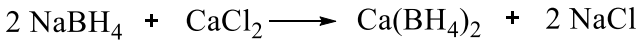
Scheme 5.8 By-product of Michael addition

To limit the formation of this undesired compound **5.15** and obtain, at the same time, a good conversion of the reaction, and, consequently, a good yield of the desired product **5.14**, an optimization of the reaction time was performed. In fact, if the reaction time is too long the formation of product **5.15** increases, meanwhile if the reaction time is not enough, a significant amount of the starting material **5.5** remains unreacted, and the yield of the product is low. We found the optimal time reaction was about 5 hours. Under these conditions, only a little spot of starting material was detected by TLC and the reaction gave the desired product with a 75% yield and a small quantity of **5.15**. However, the product can be easily purified from the other two compounds (**5.15** and **5.6**) by flash chromatography.

The second step involves the hydrogenation of the nitrile to an amine with Raney Ni and the direct cyclization to obtain the six-member ring. Raney Ni is a pyrophoric reagent so the reaction and the workup must be performed under inert atmosphere or with a suitable reactor for hydrogenation. Unfortunately, this reaction was not performed due to some problems with procuring Raney Ni.

Thus, the project was continued starting from the commercially available compound **5.6**, following the procedure shown above (*Scheme 5.6*).

The first step involves the reduction of the ester to alcohol with freshly prepared calcium borohydride (*Scheme 5.9*).



Scheme 5.9 Formation of calcium borohydride

This reagent is more reactive than sodium borohydride and can easily reduce esters in addition to aldehydes and ketones. The increased reactivity can be attributed to the major Lewis acidity of the cation, which confers increased electrophilicity on the carbonyl group (by Lewis acid-Lewis base formation). Once the reaction is complete, acidic workup and extraction with DCM were performed to hydrolyse the borate complexes and recover the alcohol, and to remove NaCl formed during the reaction. The reaction proceeds with good yield but the workup was troublesome: the product is a highly polar compound and was difficult to isolate because of its poor solubility in organic solvents and strong affinity toward water. To completely recover the product, we tried to evaporate the aqueous phase and filter the solid obtained on a sintered funnel, but we were not able to separate the product from NaCl. In addition, we observed that the next reaction, the dehydration, could not occur if impurities are present. To solve this problem, a careful optimization of the workup conditions was performed.

After some solubility tests, AcOEt was used as solvent for the extractive work up but, also in this case, only a small quantity of the product was recovered. Consequently, the aqueous phase was freeze-dried, divided into aliquots and different strategies were attempted in order to isolate the pure product.

- Firstly, a purification by flash chromatography, using AcOEt: MeOH 8:2 as eluent, was performed but we could not elute the product, even increasing the polarity of the solvent. Probably, it was not stable on silica or it remained attached to the column due to the high polarity.
- Thus, we tried to solubilize the lyophilized crude in AcOEt or iPrOH at reflux and filter on a sintered glass funnel. In both cases, only a small amount of product was obtained. In addition, using iPrOH, a gummy was obtained after filtration and additional acidic treatment was necessary to recover the product.
- Another strategy attempted was the solid-liquid continuous extraction with Soxhlet. This technique is particularly useful when the desired compound has limited solubility in a solvent, as for our product, and the impurity (in our case NaCl) is insoluble in that solvent. The main advantage of this system is that just one batch of warm solvent is used instead of performing several classical extractions. The most significant drawback is the long extraction times. The procedure was performed using $CHCl_3$ as solvent. After one week, the product was obtained with good quantity but, unfortunately, it was not pure.
- We also tried to directly subject the lyophilized compound to the next reaction, but, as we had previously observed, the reaction didn’t occur.

Finally, we accomplished a different work up procedure described in literature[11]. After the reaction, the solids were filtered and washed with MeOH. The filtrate was concentrated and Et_2O was added to precipitate a gummy material. After decantation, the gum was triturated with Et_2O and decanted. Deionized water was added to the gum, and the solid was filtered and washed. The aqueous filtrates were saturated with potassium carbonate and extracted with DCM. Unfortunately, not even this procedure was successful; in fact, we observed from TLC analysis that the product remained in the aqueous phase.

Due to these unsuccessful results, we decided to try to optimize the initial conditions. The problem was overcome performing a classical liquid-liquid extraction adding a solution of 3 N citric acid monohydrate until pH was between 2-3. It is very important to check the pH of the aqueous phase; in fact, if the pH is too acidic (pH< 2) the lactam can be opened but if the pH is not enough acid the product can't be extracted. Crude product was then stirred until all the solids had dissolved (about 10-15 minutes) and extracted with DCM: MeOH 9:1 (v/v). To recover the product several extractions are required (9-12 times) but, under these conditions, the product **5.13** was obtained in good yield (89 %) as a pure white solid without any further purification needed.

The same procedure was later applied on a big scale (4 grams) and the product was obtained with reproducible yields.

The alcohol **5.13** was next subjected to the dehydration reaction with DCC in the presence of catalytic amounts of cuprous iodide in toluene. It is well known that DCC in presence of catalytic Cu(I) efficiently dehydrates alcohols with β-substituted acceptor groups[12]. The reaction of β- hydroxy ketone affords α,β-unsaturated ketone.

During this step, difficulties were encountered with the separation of the by-products (Cu and DCU) from the target monomer. This is a problem for the next polymerization since even a small amount of these by-products can interfere with the reaction. When the dehydration reaction was performed on a small scale (about 100 mg), DCU and Cu were easily removed by filtration from Et_2O and subsequent flash chromatography and the product **5.4** was obtained with a good yield (95%). Moving on to the scale-up of the reaction (about 3 g), however, these purification procedures were not adequate, and the product was obtained impure. The presence of DCU was detected by ^{1}H-NMR spectroscopic analysis, meanwhile, it was possible to identify the presence of Cu from the colour of the product (yellow-green if contaminated, white if pure). In order to isolate the pure product **5.4**, we tried to perform, on the chromatographed compound, an aqueous extraction with a 5:1 solution of EDTA (0.05M in deionized water): MeOH to remove copper and a further filtration to remove the remaining DCU. Unfortunately, product was still not pure and both DCU and copper (the colour of product was yellow) were still present. Optimization of the

purification procedure was finally achieved using sublimation technique. In this way, the pure monomer was obtained as a white solid with a good yield (78%).

Therefore, the solubility of the monomer in different solvents was investigated. Overall, we observed that the purified monomer had good solubility in most organic solvents and was water-soluble, as indicated in *Table 5.1* below. On the contrary, the product obtained before sublimation had a poor solubility in almost all solvents.

Table 5.1 Solubility of pure 3M2Pip in various solvents

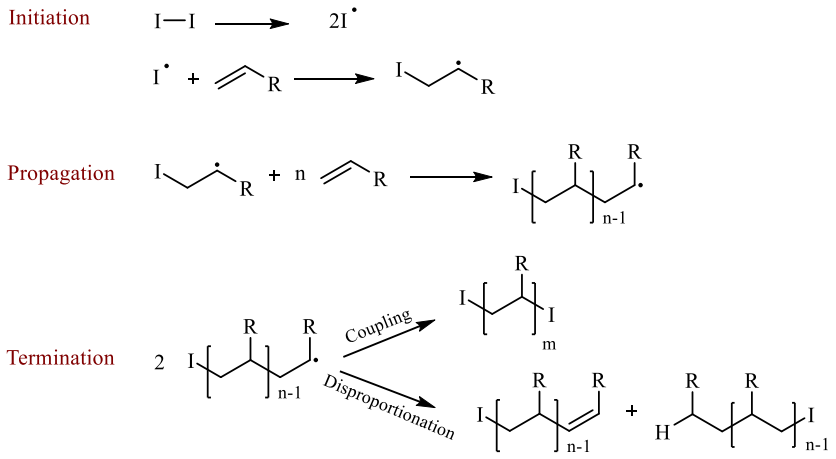
Solvent	*Solubility*	*Solvent*	*Solubility*
Acetonitrile	Ps	Diethyl ether	Is
Chloroform	S	Diethyl acetate	Is
Dichloromethane	Ps	Dioxane	S
Dimethylformamide	S	Tetrahydrofuran	Is
Dimethyl sulfoxide	S	Methanol	S
Isopropanol	Ps	Water	S

S= soluble. Is= insoluble, Ps= partially soluble upon heating

5.2.2 Study of polymerization of 3M2Pip

In the second part of this study, preliminary investigations into the free-radical polymerizations of 3M2Pip were conducted.

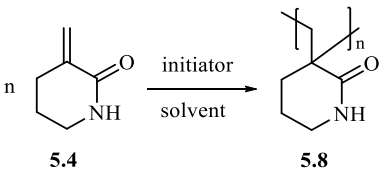
Conventional free radical polymerization is one of the most important commercial processes for the synthesis of high molecular weight polymers. The versatility of the technique derives from several advantages such as the functional group tolerance, adaptability to a wide range of reaction conditions, simplicity, and the ability to polymerize a wide range of vinyl monomers. Albeit, conventional free radical polymerization also has significant limitations as well, including poor control over molar mass and molar mass dispersity, poor control over macromolecule architecture and over copolymer composition[13]. This is ascribed to the very fast initiation and termination of the polymer chains, throughout the polymerization. Its mechanism consists of three important steps: initiation, propagation and termination, shown in *Scheme 5.10.*



Scheme 5.10 Mechanism of free radical polymerization

In the initiation step, primary radicals are generated by thermal, redox or photochemical mechanism. The initiator radicals add to the monomer to produce the propagating radicals. In the propagation step, the successive addition of monomer units to the propagating radical results in a growing polymer chain. Chain growth is terminated via bimolecular reactions either by coupling of radicals or by disproportionation between radicals.

The α-methylene-õ-valerolactam monomer can be polymerized by the free radical mechanism in the presence of a thermal initiator.



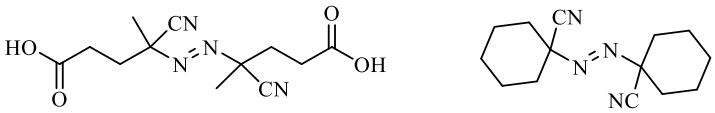
Scheme 5.11 Free radical polymerization of 3M2Pip

Different conditions of solvent, temperature and initiator were tested in order to achieve a good conversion. The choice of the initiator was done considering two factors: a) its solubility and b) its half-life ($t_{1/2}$, time required to reduce the original initiator content of a solution by 50%, at a given temperature). During the polymerization, an important aspect is to avoid the presence of oxygen that can interfere with the radical mechanism. For this reason, the reaction flask was degassed with Ar for 30 minutes or via the freeze-pump-thaw procedure (4 cycles) before backfilling with Ar.

Firstly, we decided to perform the polymerization reaction using the redox couple *t*BuOOH/Na_2SO_3. Redox initiation can be used to initiate polymerization relying on the free radicals producing in the course of oxidation-reduction reaction. The main advantage of this kind of initiators is that they can produce radicals under

mild conditions (aqueous system and low temperature) due to their lower activation energy[14]. The reaction was performed in distilled deionize water (2.5 M) using a monomer: *t*BuOOH ratio of 100:1 and *t*BuOOH: Na_2SO_3 ratio of 1.4:1. 1% DMF was also added as an internal reference to later determine the conversion of the reaction. To avoid the presence of the oxygen the reaction mixture was degassed with Ar. After 24 h the reaction was stopped by opening the flask to air and cooling in ice and the polymer was isolated by freeze-drying. With these conditions, the polymerization reaction did not occur (checked by ^{1}H-NMR spectroscopic analysis).

Due to the unsuccessful result with the redox initiator, the homopolymerization of 3M2Pip was carried out using azo compounds as initiator. Depending on the reaction temperature, two different initiators were used: 4,4'-azobis(4-cyanovaleric acid) (ACVA, 10h half-life at 69°C in water) and 1,1′-azobis(cyclohexanecarbonitrile) (ABCN, 10h half-life at 88°C in toluene). The reactions were performed with a monomer: initiator ratio of 50:1 in the minimum quantity of solvent and were monitored by ^{1}H NMR spectroscopic analysis after 24 h.



5.16, ACVA **5.17**, ABCN

Scheme 5.12 Initiators

The polymer was purified by dialysis against water (dialysis tubing with MWCO of 3 500 g/mol) and recovered by evaporation of the solvent. Preliminary results are reported in *Table 5.2.*

Table 5.2 Preliminary results of free radical polymerization

Entry	*Initiator*	*Degassing method*	*Solvent* [c]	*Temperature*	*Conversion* [a]
1	ACVA[b]	Degassed with Ar	DMSO (2 M)	80°C	23%
2	ABCN[d]	Degassed with Ar	DMF (1.5 M)	100°C	24%
3	ABCN[d]	Degassed with Ar	DMF (1.5 M)	120°C	44%
4	ACVA[b]	Freeze pump thaw	DMF (1.5 M)	75°C	51%

[a] Determined by ^{1}H-NMR, [b] ACVA: 4,4'-azobis(4-cyanovaleric acid). [c] dried over MS 3Å, [d] ABCN: 1,1′-azobis(cyclohexanecarbonitrile)

The conversion, determined by ^{1}H-NMR analysis (*Paragraph 5.4.1*) was quite low, but these preliminary results demonstrated the possibility to polymerize 3M2Pip monomer. Based on these preliminary experiments, subsequent attempts to perform

the reaction may involve the use of DMF as solvent, at 1.5 M, at temperatures progressively higher than 75 °C, with freeze pump thaw procedure. As the results show, this system is very oxygen sensitive, and, therefore, it must be treated to freeze-pump thaw procedure, which is a very efficient method of degassing, instead of merely purging with argon.

^{1}H NMR spectra of 3M2Pip and poly(3M2Pip) in D_2O are given in *Figure 5.3* and *5.4*. The disappearance of the methylene protons in the chemical shift region of 6.1-5.5 ppm and the broadening of the peaks in the lower chemical shift region are indicative of polymer formation. Integration of polymeric protons d (3.2 ppm) to protons a,b and c (2.6-1.1 ppm) was in a ratio of 2:6, further confirming the structure

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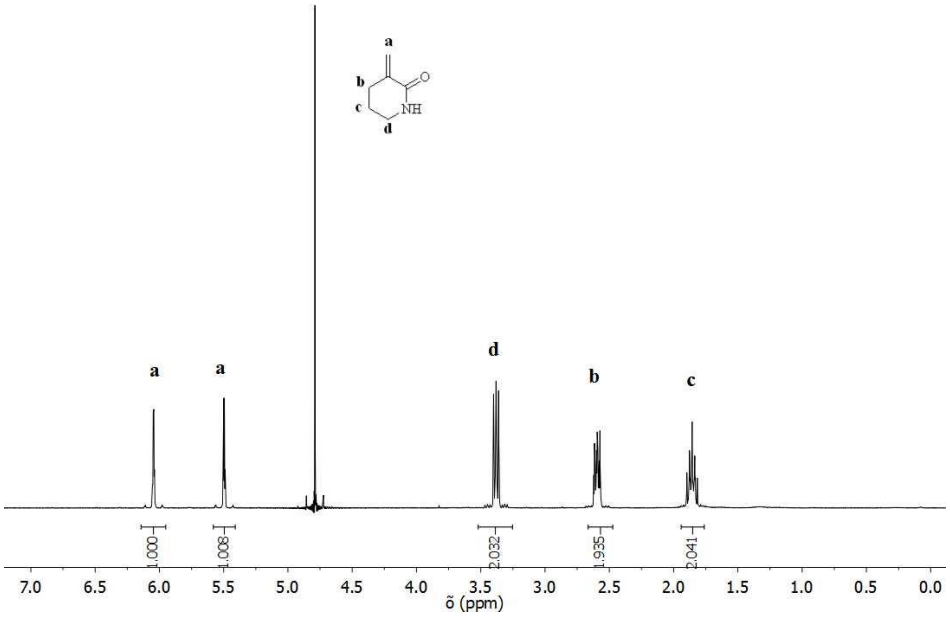


Figure 5.3 ^{1}H-NMR spectrum of monomer 3M2Pip

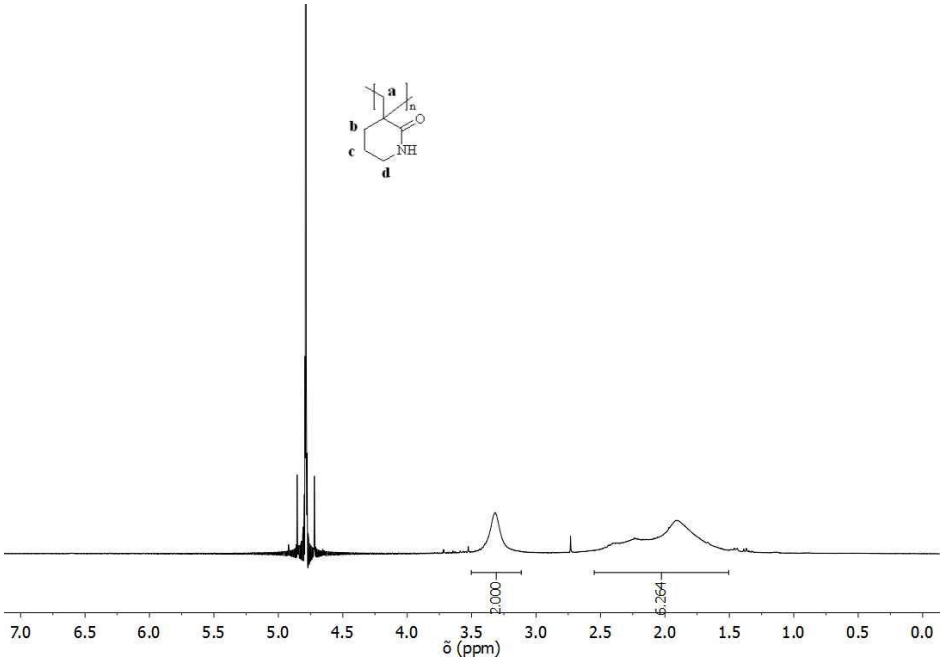


Figure 5.4 ^{1}H-NMR spectrum of poly(3M2Pip)

5.3 Conclusion and outlook

In conclusion in this study, the synthesis of monomer 3M2Pip was optimized via a two steps sequence and the desired product was obtained with high yields and on grams scale. First attempts to perform the polymerization reaction were performed and although the reaction has still to be optimized, the preliminary results are promising.

Considering the future work once obtained the lactam- based polymers they can be used as macroinitiator to prepare polyester block copolymers with well-characterized segment lengths and narrow polydispersity. To this aim, well-defined end-functionalized polymer has to be prepared.

Reversible-deactivation radical polymerization (RDRP) methods[15] are important tools for the synthesis of well-defined, end-functionalized polymers. The general concept behind RDRPs relies on suppressing termination reactions, in order to overcome the drawbacks of free radical polymerizations. Ideally, polymerisation will, therefore, continue until all the monomer has been consumed and can be restarted when more monomer is added to continue chain growth. These techniques allow the synthesis of precise and complex polymeric architectures with predictable molar masses, low molar mass dispersity as well as the introduction of various well-defined end functionalities.

Reversible addition-fragmentation chain transfer (RAFT)[16] is considered to be one of the most versatile reversible deactivation radical polymerization protocols. This polymerization method requires the usage of chain transfer agents (CTA, based on thiocarbonylthio compounds), commonly referred to as RAFT agents, to gain control over the polymerization of various monomeric structures. RAFT agents generally consist of a leaving (R) group, and a thiocarbonyl thio functional (Z) group, both retained in the final polymer as α and ω end-groups, respectively. Therefore, by suitable choice of the RAFT agent is possible to introduce the desired well-defined end-groups in the polymer[17].

Therefore, in a continuation of the work, the monomer will be polymerized by controlled polymerization methods, like RAFT mediated polymerization, in order to target the synthesis of i.e. HO-P(3M2Pip) to be subsequently used for the synthesis of polyester second blocks. These polymers can find useful applications in drug delivery systems.

5.4 Experimental section

5.4.1 General remarks

All chemicals were reagent-grade and purchased from commercial sources. DCC was purified by solubilisation in DCM and filtration to eliminate the by-product DCU. The solid obtained was checked by ^{13}C-NMR spectroscopy and dried under vacuum for one night before using.

DCM, DMSO and DMF were kept over activated molecular sieves. EtOH was dried by stirring over powdered KOH for one night; then it was distilled and kept over molecular sieves 3 Å. MeOH was distilled over Mg/I_2 and kept over molecular sieves 3 Å. Toluene was distilled and kept over molecular sieves 3 Å. Distilled, deionized water was obtained from a Millipore Milli-Q purification system.

Moisture and oxygen sensitive reactions were carried out under an inert atmosphere using argon gas.

TLC analyses were carried out on silica gel plates and viewed at UV (254 nm) and developed with Hanessian stain (dipping into a solution of $(NH_4)_4MoO_4{\cdot}4H_2O$ (21 g) and $Ce(SO_4)_2{\cdot}4H_2O$ (1 g) in H_2SO_4 (31 mL) and H_2O (469 mL) and warming) and $KMnO_4$ (dipping into a solution of 1.5 g of $KMnO_4$, 10 g K_2CO_3, and 1.25 mL 10% NaOH in 200 mL of water and warming). *Rf* values were measured after an elution of 7-9 cm.

All the products were characterized by *NMR spectroscopy*. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a 300 MHz Varian VNMRS or 400 MHz Varian Unity Inova spectrometer with TMS (^{1}H-NMR: 0.000 ppm) or the central peak of $CDCl_3$ (^{13}C: 77.160 ppm) as internal standard. Compounds were dissolved in $CDCl_3$, D_2O or DMSO-d_6. Chemical shifts are reported in ppm (δ scale). Coupling constants are reported in Hertz. Resonances are described as s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet) and m (multiplet) or combinations thereof.

Column chromatographies were done with the "flash" methodology using 400-730 mesh silica.

5.4.1.1 Degassing method for polymerization reactions

Two main methods have been applied to degas the reaction environment and remove the oxygen. The first one involves the careful purging of the reaction wit Ar for 30 minutes. In the second method, the freeze-pump-thaw procedure, a solvent in a sealed Schlenk flask is frozen by immersion of the flask in liquid N_2. When the solvent is completely frozen, the flask is opened to high vacuum and pumped 5-10 minutes, with the flask still immersed in liquid N_2. The flask is then closed and warmed (dipping the flask in an acetone bath can accelerate the process) until the solvent has completely melted. During this procedure gas bubbles visibly evolve from the solvent. This process is repeated (usually three, four times) and after the last cycle the flask is backfilled with the inert gas.

5.4.1.2 Polymer isolation

The polymer was first dialysed, to remove impurities, before freeze drying to remove solvent and isolate the solid polymer.

Dialysis consists of placing the polymer in a porous tube (SnakeSkin® dialysis tubing), with a molecular weight cut-off of 3500 g/mol to enable diffusion of low molecular weight contaminants out of the tube with water entering the tube to compensate for the high osmotic pressure caused by the polymer. Polymerized samples were generally dialyzed for 16-24 h, changing the water every 6 hours. Water was removed from the purified polymer by freeze-drying.

5.4.1.3 Determination of monomer conversion

The monomer conversion was determined by ^{1}H-NMR spectroscopy from monomer and polymer signals. Conversion is defined as the ratio between the hydrogens of polymer and the sum of the hydrogens of monomer and polymer.

$$C\% = \frac{H\ polymer}{H\ polymer + H\ monomer}\ x\ 100$$

Considering the NMR spectrum, as the monomer is consumed, the methylene protons are converted, and the peaks become smaller. Therefore, the degree of conversion can be estimated by comparing the integration of the methylene protons (at 6.0 ppm) of the monomer to the broad backbone peak of the polymer.

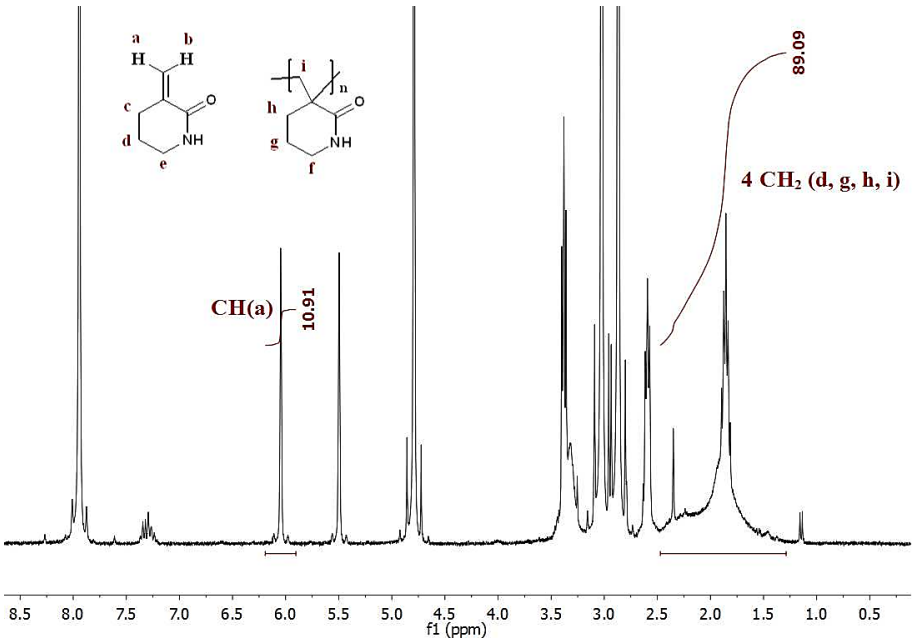


Figure 5.5 Determination of conversion from ^{1}H-NMR spectrum

As shown in *Figure 5.5* the peak between 2.45 and 1.30 ppm involves 3 CH_2 of the polymer backbone (h, g, i) but also a further CH_2 of the monomer (d). Therefore, in order to calculate the conversion, the integration of H monomer and of H polymer to insert in the equation reported above, is given by the following equations:

$$H\ monomer = I\ a = 1H$$

$$H\ polymer = I\ g,h,i,d - (Ia \times 2) = \frac{6H}{6} = 1\ H$$

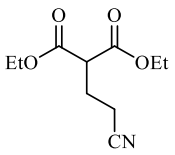
where I is the peak integration.

To better explain a practical example is shown below.
H monomer = 10.91 = Ia
H polymer = I (g,h,i,d) - (Ia x2) = 89.09 – (10.91 x 2) = 89.09 – 21.82 = 67.27

$$\text{Conversion (\%)} = \frac{67.27/6}{67.27/6 + 10.91/1} = \frac{11.212}{22.122} \text{ x } 100 = 51\%$$

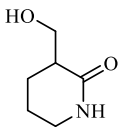
5.4.2 Experimental procedures

5.4.2.1 Synthesis of 3M2Pip monomer



Diethyl 2-(2-cyanoethyl)malonate 5.14
A solution of diethyl malonate (236 μL, 1.56 mmol) in dry EtOH (1.6 mL) was cooled to 0°C under Ar atmosphere. EtONa (1 M, 1.6 mL, prepared in situ from Na (113 mg, 4.91 mmol) and EtOH dry (4.9 mL)) was added and the mixture was stirred for ten minutes. After ten minutes, acrylonitrile (102 μL, 1.56 mmol) was added and the reaction was stirred at room temperature for 5 h. Then, EtOH was evaporated. The reaction mixture was poured in 10 mL of NH_4Cl (sat. sol.) and extracted with DCM (3 x 10 mL). The organic phase was washed with brine (10 mL), dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography on silica gel (hexane: AcOEt 9:1 and hexane: AcOEt 8:2) to give 246 mg (75% yield) of **5.14** as a colourless oil.

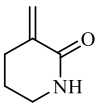
5.14, colourless oil, *Rf* = 0.38 (hexane: AcOEt 8:2), developed with $KMnO_4$.
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 4.16 (q, J = 7.1 Hz, 4H, 2 C$\underline{H_2}$CH$_3$), 3.44 (t, J = 7.2 Hz, 1H, CH), 2.44 (t, J = 7.3 Hz, 2H, C$\underline{H_2}$CN), 2.18 (q, J = 7.2 Hz, 2H, CHC$\underline{H_2}$), 1.22 (t, J = 7.1 Hz, 6H, 2 CH$_2$C$\underline{H_3}$).
The analytical data conform to those reported in literature[18].



3-(Hydroxymethyl)piperidin-2-one 5.13
A stirred suspension of ethyl 2-oxopiperidine-3-carboxylate **5.7** (4 g, 23.36 mmol) and anhydrous $CaCl_2$ (2.593 g, 23.36 mmol) in dry MeOH (40 mL)

under Ar atmosphere was cooled at 0°C. $NaBH_4$ (1. 768 g, 46.73 mmol)) was added and the reaction was stirred for 2 h at 0°C. Then the reaction was allowed to reach room temperature and stirred overnight. MeOH was evaporated under vacuum. 3 N aqueous citric acid monohydrate solution was added in small portions and stirred for 10 minutes until all solid material was dissolved (pH = 2/3). The solution was extracted with CH_2Cl_2: MeOH 9:1 (12 x 55 mL), dried ($MgSO_4$) and concentrated. The pure product **5.13** (2.676 g, 89% yield) was obtained as a white solid and used for the next reaction without any further purification.

5.13, white solid, *Rf* = 0.42 (AcOEt: MeOH 8:2), developed with Hanessian stain.
^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.58 (s, 1H, NH), 4.18 (s, 1H, OH), 3.85 – 3.53 (m, 2H, C$\underline{H_2}$OH), 3.28 (m, 2H, C$\underline{H_2}$NH), 2.58 – 2.32 (m, 1H, C$\underline{H}$), 1.97 – 1.82 (m, 2H, C$\underline{H_2}$CH$_2$NH), 1.82 – 1.67 (m, 1H, C$\underline{H_2}$CH), 1.57 – 1.40 (m, 1H, C$\underline{H_2}$CH). *^{13}C NMR* (75 MHz, $CDCl_3$, 25 °C, TMS) δ = 175.76 (C=ONH), 64.40 ($\underline{C}H_2OH$), 42.76 ($\underline{C}H$), 42.17 ($\underline{C}H_2NH$), 23.66 ($\underline{C}H_2CH$), 21.86 ($\underline{C}H_2CH_2NH$).
The analytical data conform to those reported in literature[9b].



3-Methylenepiperidin-2-one (3M2Pip) 5.4

Synthesis on a small scale

DCC (160 mg, 0.77 mmol) was added to a stirring solution of alcohol **5.13** (77 mg, 0.59 mmol) in dry toluene (1 mL), under Ar atmosphere. The reaction was heated at 110°C and CuI (10 mg, 0.05 mmol) was added and the mixture was stirred at reflux for 2 h. Then, the reaction was cooled to room temperature and deionized water (1 mL) was added and stirring continued for 1 h. Et_2O (2 mL) was added and the reaction was filtered in order to remove DCU. The solution was extracted with CH_2Cl_2 (3 x 5 mL), dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography on silica gel (AcOEt) to give 63 mg (95% yield) of **5.4** as a pale yellow solid.

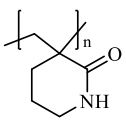
Synthesis on a larger scale

DCC (4.642 g, 22.50 mmol) was added to a stirring solution of alcohol **5.13** (2.2352 g, 17.31 mmol) in dry toluene (27 mL), under Ar atmosphere. The reaction was heated at 110°C and CuI (297 mg, 1.56 mmol) was added and the mixture was stirred at reflux for 2 h. Then, the reaction was cooled to room temperature and deionized water (25 mL) was added and stirring continued for 1 h. Et_2O (50 mL) was added and the reaction was filtered in order to remove DCU. The solution was extracted with CH_2Cl_2 (12 x 40 mL), dried ($MgSO_4$) and concentrated. The residue was purified by flash column chromatography on silica gel (AcOEt: MeOH 15:1) followed by sublimation for 6 hours to give 1.504 g (78% yield) of **5.4** as a white solid.

5.4, white solid, *Rf* = 0.67 (AcOEt: MeOH 5:1, UV).
^{1}H NMR (300 MHz, $CDCl_3$, 25 °C, TMS) δ = 6.50 (s, 1H, NH), 6.24 – 6.05 (m, 1H, C$\underline{H_2}$=C), 5.35 – 5.16 (m, 1H, C$\underline{H_2}$=C), 3.32 (ddd, J = 7.4, 4.2, 1.8 Hz, 2H, C$\underline{H_2}$NH),

2.51 (ddt, J = 7.9, 4.5, 1.6 Hz, 2H, C$\underline{H_2}$C=), 1.84 – 1.74 (m, 2H, C$\underline{H_2}$CH_2NH). The analytical data conform to those reported in literature[9b].

5.4.2.2 General procedure for conventional radical polymerization of 3M2Pip



Homopolymerizations of 3M2Pip **5.4** were carried out in a 25 mL pear flask, where 3M2Pip (150 mg, 1.35 mmol for *Entries 1 and 2*, 50 mg, 0.45 mmol for *Entries 3 and 4, Table 5.2*) and AIBN or ACVA, in a ratio of 50:1 were added. Homopolymerizations were carried out in DMSO or DMF as solvent. Subsequently, the polymerization mixture was degassed with argon gas for 30 min or subjected to the freeze-pump-thaw procedure. The pear-shaped flask was placed in an oil bath for 24 h. Reaction temperatures are specified in *Table 5.2*. The polymerization was stopped by opening the flask to air and allowing to reach room temperature. The polymer was isolated by dialysis against water for 24 h, replacing the water every 6 h and subsequent evaporation of the solvent.

5.5 Bibliography

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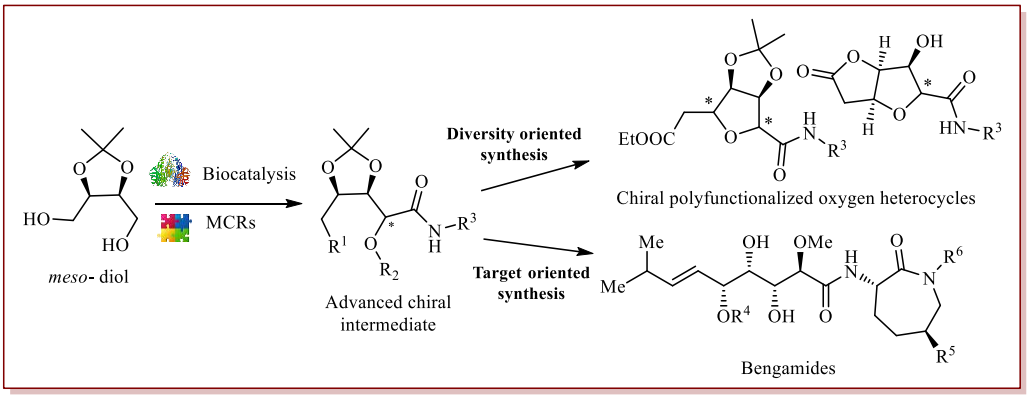
SUMMARY

In the progression of modern society, the inevitable exhaustion of fossil resources becomes an increasingly concerning matter. These resources are not only the basis of the energy production of the world but also the precursor for many important platform chemicals. Therefore, development of green and renewable alternatives to the chemicals used in the current industry has become necessary[1]. From a chemical point of view the most important renewable resource for the synthesis of platform chemicals is lignocellulosic biomass since it is an economical, and widely available carbon source, apart from oil and coal. In addition, it is non-edible by humans and, consequently, its use avoids the undesirable competition between food and chemicals[2] (**Chapter 1**). Within this context, the aim of the present thesis was the development of new efficient methodologies for obtaining high added-value products starting from renewable sources. The bio-based building blocks obtained were subsequently employed for the synthesis of polyfunctionalized chemicals (**Part A***)* and of polymers (**Part B**).

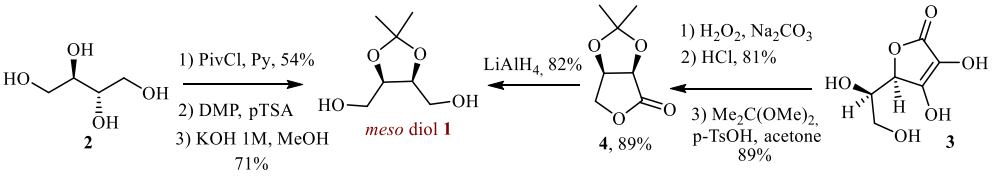
The first part of the thesis (**Part A**, *Chapters 2, 3, 4*) describes the research carried out within the BioOrganic Chemistry Group at the University of Genova. It focuses on the utilization of a bio-based *meso* diol for the synthesis of sugar derived structures, through the coupling of biocatalysis and multicomponent reaction (MCRs). These two different approaches are able to satisfy the requirements of an ideal approach, regarding selectivity (stereocontrol), efficiency and sustainability (*Paragraph 2.4*)[3]. MCRs can afford higher molecular complexity in just one step, achieving at the same time atom and step economy with high tolerance of different functional groups. In addition, they usually need mild conditions and they are generally compatible with "green solvents"[4]. However, a major challenge in MCR chemistry is the overall poor stereocontrol in most of these reactions. Within this context, biocatalysis is an important tool since it is an efficient, selective and ecofriendly strategy for the enantiocontrolled synthesis of chiral substrates, which can be used to direct the stereochemical outcome of MCRs. The combination of biocatalytic principles with MCRs strategies would represent a highly desirable approach towards the green stereoselective synthesis of chiral, bio-based, densely functionalized compounds[5].

The main scope of Part A of the present thesis was, therefore, to further investigate this field. In particular, my work focused on the synthetic elaboration of the chiral molecules obtained through biocatalysis to give enantiomerically pure building blocks to be used in diastereoselective Passerini reactions. In order to further establish the synthetic usefulness of this new method, we demonstrated its application to the diversity-oriented synthesis of chiral, bio-based, oxygen heterocycles and to the target-oriented synthesis of Bengamides.



Scheme 1 Coupling of biocatalysis and MCRs for the synthesis of chiral, bio-based, densely functionalized compounds

As stated before, common ground for these two studies is the bio-based compound, *meso* diol **1**, derived from erythritol[6] **2** or D-isoascorbic acid **3**, both obtainable from biomass. The first part of my project was, therefore, focused on the synthesis of this compound (*Paragraph 2.5*).

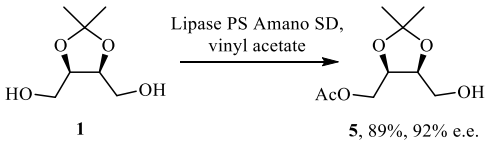


Scheme 2 Synthesis of *meso* diol

In particular, a first period was dedicated to the optimization of the work up conditions of a procedure reported in literature[7] and already used in the research group[8], starting from erythritol **2**. Two attempts for isolating the product **1** has been accomplished: the use of a strongly acid cation exchange resin (Amberlite® IR 120 hydrogen form) to neutralize KOH, and a liquid-liquid continuous extraction. Both procedures presented some problems and afforded the product with a comparative yield of the reported method. The second strategy for the synthesis of diol uses D-isoascorbic **3** acid as starting material. The first two steps to obtain compound **1** were performed following a procedure reported in literature[9], meanwhile a brief optimization of the reduction step occurred. This second approach has the main disadvantage of starting from a chiral substrate, D-isoascorbic acid, to afford an achiral product, the diol, which will be used for the synthesis of chiral building blocks in a later step of the project. On the other hand, this disadvantage is compensated for by the better yields obtained in the various steps.

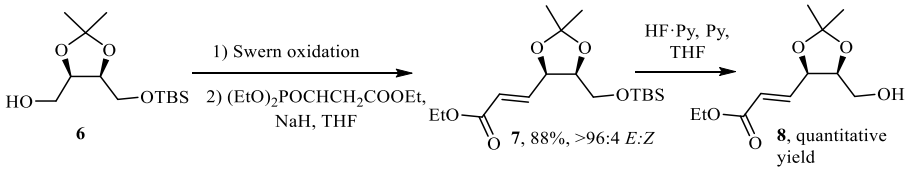
The first application of the combination of biocatalysis and MCRs starting from *meso*-diol was directed to the synthesis of chiral, bio-based, oxygen heterocycles scaffolds (**Chapter 3**).

Diol **1** is a symmetrical molecule, therefore it is not optically active. To access enantiomerically pure building blocks, it was subjected to lipase-mediated desymmetrization, following an optimized protocol previously developed by our group[8a] and gave the desired product **5** in high yield and e.e.(*Paragraph 3.2.1*).



Scheme 3 Enzymatic desymmetrization of diol

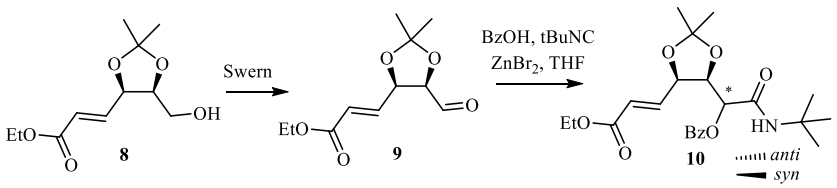
Then, manipulation of the enantiomerically pure building block **5,** in order to introduce a double bond that can be later used in cyclization reactions, was investigated (*Paragraph 3.2.2*). The chiral alcohol was oxidized to the corresponding aldehyde under Swern conditions and the crude was directly subjected to the following olefination, employing Horner-Wandsworth-Emmons conditions. Firstly, the reaction was performed on compound **5** but due to difficulty on removing the acetyl group in a later step of the synthesis, the reaction was, then, carried out on compound **6** which presents a TBS group. This group can be introduced into the monoacetate through a protection-deprotection sequence. Removal of the silyl ether was not as simple as we expected. After an extensive optimization we found the best conditions in the use of HF·pyridinium (35 eq) complex in the presence of pyridine.



Scheme 4 Manipulation of functional groups

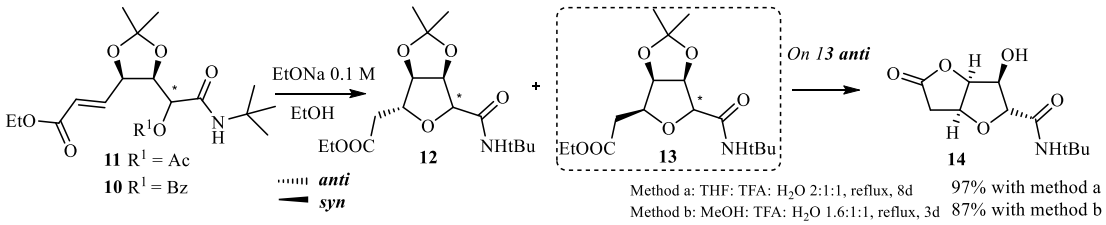
With this functionalized molecule available, its use in the Passerini reaction as oxo-compound (with previous oxidation of alcohol functionality) was extensively studied (*Paragraph 3.2.3*). To achieve a better stereoinduction, a Lewis-acid ($ZnBr_2$) mediated process was performed[8]. To avoid the overoxidation of aldehyde we decided to perform the oxidation reaction under Swern conditions. Passerini reaction was firstly studied using acetic acid and *t*BuNC but the d.r. was difficult to determine since the products were not separable by HPLC analysis. In addition, we observed the formation of truncated Passerini products. The reaction was finally optimized using

benzoic acid (1.1 eq), *t*BuNC (1.1 eq) and $ZnBr_2$ (0.4 eq) at 20°C in dry THF, affording the desired products with 56% yield and d.r. 76:24.



Scheme 5 Diastereoselective Passerini reaction

The configuration of the new-formed stereogenic centre was determined at a later stage of the study, by ^{1}H-NMR analysis on a cyclic derivative (*Paragraph 3.2.4.2*). With these conditions, a small library of Passerini adducts was synthesized. The yields were moderate (from 46% to 78%) meanwhile the diastereoselective ratio was in all cases good, and even higher than for the first model compound, ranging from 77:23 to 96:4. Finally, in order to broaden the scaffold diversity, the cyclization of the erythritol-derived Passerini products was explored quite extensively (*Paragraph 3.2.4*).



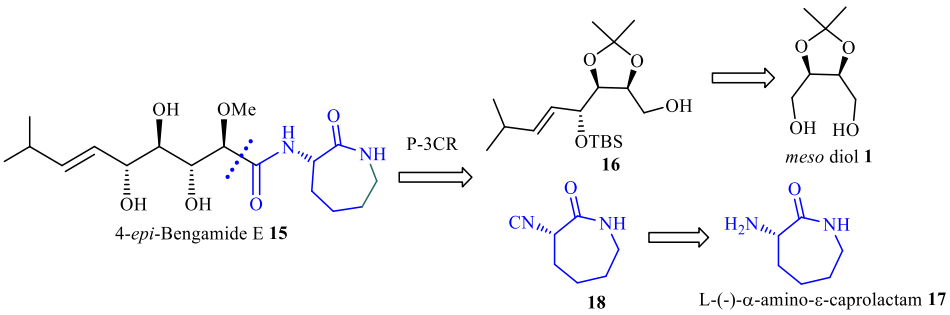
Scheme 6 Intramolecular Michael addition and synthesis of bicyclic lactone

Intramolecular Michael addition, performed on both the diastereoisomers of Passerini product, afforded tetrahydrofuran with good yields (from 53% to 83 %) and high d.r. (from 83:17 to 92:8). Lactone **14** was obtained from tetrahydrofuran **12a** upon acidic removal of the acetonide protecting group followed by spontaneous lactonization. We also attempted to perform a ring closing metathesis taking advantage of the α,β-unsaturated ester and of a double bond introduced on Passerini product using a suitable carboxylic acid. The reaction was performed on the corresponding protected allylic alcohol as well but, unfortunately, the obtainment of the 9 membered unsaturated lactones was unsuccessful.

The general synthetic approach involving the coupling of bicatalysis and MCR was also applied to the target oriented synthesis of Bengamides, a wide family of natural products of marine origin (**Chapter 4**).

Through a retrosynthetic analysis, a novel strategy for the total synthesis of Bengamides, and in particular of 4-*epi*-Bengamide E **15**, has been developed by us. In

particular, the general structure of this compound can be obtained through a Passerini reaction between the chiral alcohol **16** derived from *meso* diol **1**, on which the olefinic residue is introduced, and the aminocaprolactamic isocyanide **18** derived from the commercially available L-(-)-α-amino-ε-caprolactam **17**.

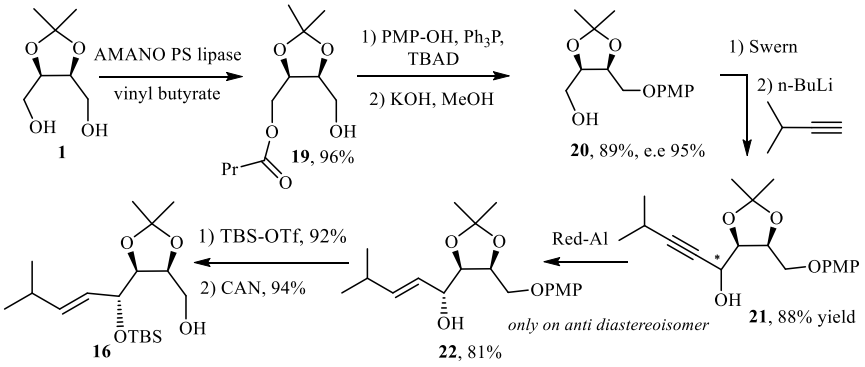


Scheme 7 Retrosynthetic analysis of 4-*epi*-Bengamide E

The main advantages of this strategy are the possibility to use a renewable source as starting material and the flexibility and convergence of the approach. Bengamide analogues can be obtained modifying caprolactamic unit and the olefinic residue by suitable use of different reagents.

Therefore, firstly my work focused on the synthesis of the substrates necessary to later performed Passerini reaction.

The synthetic sequence to obtain chiral alcohol **16** starting from *meso*-diol **1** involves five key steps.

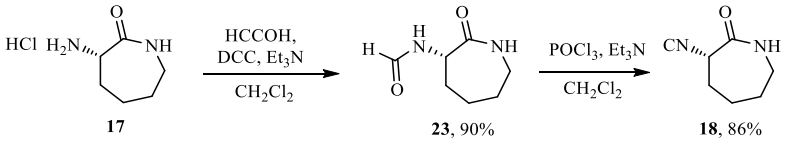


Scheme 8 Steps for the synthesis of chiral alcohol 16

In the first step a lipase-mediated desymmetrization reaction with vinyl butyrate was performed, following a procedure reported in literature[8a]. Then, compound **19** was subjected to the Mitsunobu reaction to introduce the *p*-methoxyphenyl (PMP) group and the subsequent cleavage of the ester group. The change of functional group is necessary to obtain the desired monomer because of the

instability of the acyl group in the subsequent reactions, as a previously study had demonstrated. Thus, PMP was chosen as protecting group since it has orthogonally cleavage conditions respect to the other protecting groups present on the molecule (*Paragraph 4.2.1*). Alcohol **20** was oxidized under Swern conditions and the corresponding aldehyde was subjected to the 1,2- addition of the acetylide (*Paragraph 4.2.2*). A careful optimization of this procedure was necessary to make it efficient and reproducible. In fact, the corresponding aldehyde decomposes rapidly once it is dried and tends to remain in the hydrated form and, therefore, the presence of water within the reaction environment affects the success of the subsequent reaction. Once we obtained compound **21** (88% yield, d.r: 57:43 *anti*: *syn*), we decided to improve the d.r. by oxidation with Dess Martin periodinane and stereoselective reduction of the resulted ketone with K-selectride, affording the product with a d.r. of 76:24. Propargyl alcohol **21** was, then, reduced to alkenes with sodium bis(2-methoxyethoxy)aluminium hydride (Red-Al) in dry THF at reflux to afford the double bond with *E* configuration (*Paragraph 4.2.3*). Finally, the primary hydroxy group of compound **22** was protected with TBS group and PMP protecting group was removed, using the reagent ammonium cerium (IV) nitrate (CAN). This step is particularly delicate because the acid environment of the reaction can also promote the release of the silyl group. For this reason, the reaction must take place in a very short time, about 10-15 minutes, and it is extremely important to block it promptly. The two last steps were performed only on the *anti* diastereoisomer, since it has the absolute and relative configuration of the final product.

Isocyanide **18** was synthesized starting from commercially available L-(-)-α-amino-ε-caprolactam via two steps sequence (*Paragraph 4.2.4*). The first step involves the formation of the formamide **23** through the coupling reaction with formic acid, in presence of DCC and Et_3N. Then, the dehydration reaction was carried out with $POCl_3$ to obtain the final product **18**. Particular attention was dedicated to the control of the preservation of optical purity of the product.

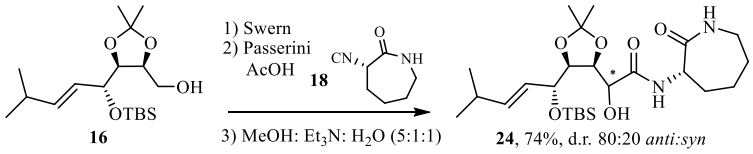


17 **23**, 90% **18**, 86%

Scheme 9 Synthesis of isocyanide 18

The synthetized building blocks, chiral alcohol **16** and isocyanide **18** were subsequently used, with AcOH, to accomplish, after Swern oxidation to aldehyde, the diastereoselective Passerini multicomponent reaction (*Paragraph 4.2.5*). The reaction was performed in a tandem procedure with Swern oxidation and the subsequent deacetylation reaction to avoid the chromatography of the Passerini products (that are difficult to separate) and to directly determine the d.r. on the final products and, thus, not having the interference of the truncated Passerini products on the determination.

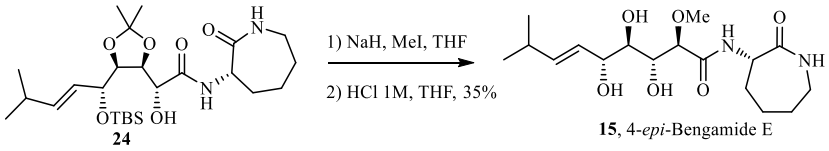
After a brief optimization we found the best condition for the Passerini reaction in the use of iPr_2O as solvent, 2 eq of isocyanide and 2 eq. of acetic acid.



Scheme 10 Diastereoselective Passerini reaction

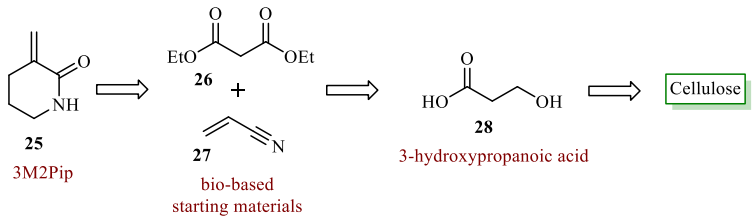
To improve the diastereoisomeric ratio we decided to investigate the possible beneficial effect of catalytic amount of $ZnBr_2$ but it did not lead to a significant improvement on the observed diastereoselectivity.

The two last steps to obtain 4-*epi*-Bengamide E involve the methylation reaction and the final cleavage of protecting groups (*Paragraph 4.2.6*). After an extensively optimization, methylation reaction was carried out using NaH and MeI at 0°C in THF. To avoid the formation of side- products, the *N*-methylated compound and the bis-methylated, it is important to conduct the reaction with a slightly excess of NaH. The final cleavage was accomplished under acidic conditions.



Scheme 11 Methylation reaction and final cleavage

The second part of the thesis (**Part B**, *Chapters 5*), focuses on the project developed in the Free Radical Group at Stellenbosch University (South Africa). The secondment was included in the Project "Synthesis, Characterization, Structure and Properties of Novel Biodegradable Polyesters (BIODEST)" that is funded under the RISE (Research and Innovation Staff Exchange) Scheme (H2020-MSCA-RISE-2017-778092)[10] (*Paragraph 5.1.1*). The project I worked on involved the synthesis and polymerization of 3-methylene-2-piperidone (3M2Pip) monomers, targeting the synthesis of well-defined hydroxyl end functional P(3M2Pip), i.e. HO-P(3M2Pip). Once obtained end-functionalized 3M2Pip-based polymers we will use them as a macro-initiator for the synthesis of degradable polyester second blocks, applicable in drug delivery polymer materials. 3M2Pip monomer is of particular interest; in fact, it can be obtained from the reaction between diethyl malonate and acrylonitrile, bio-based compounds derived from 3-Hydroxypropionic acid (3-HP), a cellulose C3 building blocks derivative[11] (*Paragraph 5.1.3*).

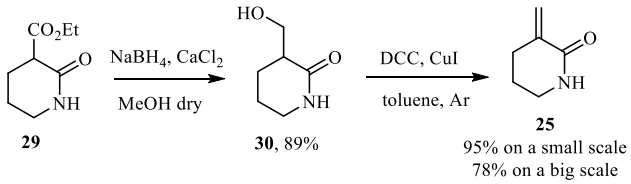


Scheme 12 3M2Pip from bio-based starting materials

My work was mainly focused on two objectives:

1) Optimization of the synthesis of 3M2Pip monomer. In particular, the main part of my work was dedicated to this step.
2) Preliminary study of polymerization of the 3M2Pip via conventional free radical mechanism.

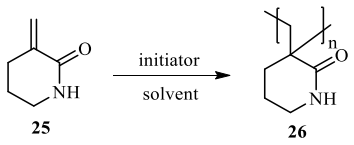
In a previous study conducted in Klumperman's group[12] the desired monomer had been synthetized starting from commercially available 2- piperidone via a 4-steps sequence. The main problems of this approach were the low yields of the two last steps. Therefore, the first aim accomplished consisted in the optimization of this synthesis. In particular, a new strategy was developed starting from the six-member ring with the ester moiety (*Paragraph 5.2.1*).



Scheme 13 New synthesis of 3-methylene-2-piperidone

The first step involves the reduction of the ester to alcohol with freshly prepared calcium borohydride. The reactions proceed with good yield, but the product is difficult to isolate because of its poor solubility in organic solvents and strong affinity toward water. The problem was overcome by addition of a citric acid solution until pH 3. Then the crude was stirred until all the solid was dissolved and extracted with DCM: MeOH 9:1. The alcohol was next subjected to the dehydration reaction with DCC in the presence of catalytic amounts of cuprous iodide in toluene. Difficulties were encountered with the separation of the by-products, Cu and DCU, from the target monomer. An optimization of the purification procedure was achieved using sublimation technique. In this way, the pure monomer was obtained as a white solid. Both the two steps of the synthesis above gave good results in terms of yield and they were applied in large scale (4 g).

In the second part of the secondment, preliminary polymerizations were conducted via conventional radical polymerization conditions[13] using 4,4'-azobis(4-cyanovaleric acid) (ACVA) or 1,1′-azobis(cyclohexanecarbonitrile) (ABCN) as the thermal initiator, with a monomer to initiator ratio of 50:1 (*Paragraph 5.2.2*).



25 **26**

Scheme 14 Polymerization of 3M2Pip

Polymerizations were carried out under an inert Ar atmosphere using DMF, DMSO or 1,4-dioxane as solvent, at different temperatures (70°C, 80°C, 100°C and 120°C). After 24 h and the polymer was purified by dialysis against water (dialysis tubing with MWCO of 3 500 g/mol) and then recovered by evaporation of the solvent. The conversion, determined by ^{1}H-NMR analysis(*Paragraph 5.4.1*) was quite low (ranging from 23% to 51%), but these preliminary results demonstrated the possibility to polymerize 3M2Pip monomer. In particular, best results were obtained using DMF (1.5 M) as solvent at 75°C (with ACVA initiator) or 120°C (with ABCN initiator).

In conclusion, with this thesis different applications of bio-based building blocks derived from renewable sources in organic synthesis and materials science have been developed, with the aim of enhancing their value by increasing complexity. These starting materials represent a plausible replacement of their petrol-based analogues, hereby laying parts of the groundwork for a more sustainable and renewable future.

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